Original Article

Association of heavy metals and trace elements in carcinoma urinary bladder: A case-controlled study

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ABSTRACT

Introduction: Abnormal levels of heavy metals (HM) and trace elements (TE) affect body metabolism and can induce carcinogenesis. This study aims to evaluate the role of HM and TE in carcinoma urinary bladder (CAUB).

Methods: Patients with biopsy-proven CAUB (n = 100) were taken as the study group, while age-and sex-matched healthy volunteers were taken as control (n = 100). Blood and urine samples were compared for Arsenic (As), Copper (Cu), Manganese (Mn), Selenium (Se), Cadmium (Cd), Lead (Pb), and Mercury (Hg) levels. Serum glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and lipid peroxidation (LPO) levels were assessed to know the redox status between the two groups.

Results: A significantly higher blood level of As, Mn, and Pb was observed in CAUB cases as compared to controls. Blood Se level was significantly lower in CAUB patients. On comparing urinary levels, CAUB patients had a higher As, Mn, and Pb levels compared to controls. Further, 68% and 59% of patients had their blood and urinary HM and TE levels above the permitted level, respectively. CAUB cases also had a lower GSH-Px (113.5 ± 44.7 vs. 163.9 ± 120.5, P = 0.0002), lower SOD levels (11.35 ± 5.6 vs. 13.75 ± 3.9, P = 0.008), and a higher LPO levels (15.5 ± 14.7 vs. 11.18 ± 11.2, P = 0.02) in the serum.

Conclusions: A significantly higher concentration of As, Mn, and Pb was noted in the blood and urine of CAUB patients compared to controls. CAUB cases also had lower serum GSH-Px and SOD levels with a concomitant increased serum LPO assay suggesting underlying oxidative stress.

INTRODUCTION

The urinary bladder is responsible for the storage of highly concentrated urine for several hours. The toxic filtrates from the kidneys get continuously deposited in the bladder, and hence, the bladder is vulnerable to various toxins, including heavy metals (HM) and trace elements (TE). HM and TE induce oxidative stress and macromolecular injuries (DNA and protein damages), leading to different types of genetic, sub-cellular, cellular, and tissue damage.[1] This oxidative damage can cause alteration in mitotic activity of dividing cells and hence, induction and progression of carcinoma urinary bladder (CAUB).[2]

TE such as Arsenic (As), Copper (Cu), Manganese (Mn), Selenium (Se), Cadmium (Cd), Lead (Pb), and Mercury (Hg) are found naturally in the environment, and humans are

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exposed to these elements through various sources such as air, food and water consumption. Studies evaluating HM and TE exposure are important as they are potentially modifiable risk factors in preventing carcinogenesis. Some studies have evaluated the role of TEs in CAUB patients with heterogeneous results.[3,4] Studies evaluating the role of HM exposure and oxidative stress in CAUB are limited. We aimed to analyze the blood and urinary concentrations of HM and TE (As, Cu, Mn, Se, Cd, Pb, and Hg) in CAUB patients in a case-control study. These elements were selected for the current study since they were the most common HM pollutants which cause deleterious effects on health and can lead to carcinogenesis.[5-8]

MATERIALS AND METHODS

Study design

The study was conducted at a single tertiary care center between April 2020 and March 2022 after obtaining ethical approval (Ref No. IEC-321/May 03, 2019). The study group included patients with CAUB confirmed histologically on transurethral resection of bladder tumor or radical cystectomy. For subgroup analysis, CAUB cases were further stratified according to grade and invasiveness into low- and high-grade disease and noninvasive (Ta) and invasive (≥T1) disease. Patients with chronic kidney disease stage 3 or more and chronic liver disease were excluded from the study. Age and sex-matched healthy volunteers were taken as controls. Written informed consent was taken from all study participants.

Study outcomes

The primary outcome measure was to compare the blood levels of HM and TE (Arsenic [As], Copper [Cu], Manganese [Mn], Selenium [Se], Cadmium [Cd], Lead [Pb], Mercury [Hg]) in CAUB patients and controls. Secondary outcome measures include a comparison of urinary concentration of HM and TE and serum antioxidant enzymes (glutathione peroxidase [GSH-Px], superoxide dismutase [SOD]) activities, and levels of lipid peroxidation (LPO) between the two groups.

Sample collection and heavy metals and trace elements evaluation

Ten milliliters of peripheral blood samples were collected in metal-free, sterile, ultra-pure ethylenediaminetetraacetic acid coated vials, whereas urine samples (10 mL) were collected in sterile metal-free vials. All samples were subjected for quantification of As, Cu, Mn, Se, Cd, Pb, and Hg using inductively coupled plasma mass-spectroscopy (ICP-MS) (ICP-MS-7800, Agilent Technologies, USA). The calibrations, quality control, and interpretation of results were followed as per our prior study on HM and TE in renal cell carcinoma.[9] All the blood samples in cases and controls were taken in the morning after overnight fasting. The blood and urine samples of CAUB cases were collected before the tumor resection/removal.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation and compared with Student's *t*-test. Categorical variables were expressed as frequency (percentage) and compared with Chi-square or Fischer's exact test. Statistical analyses were performed using GraphPad® Prism 5 (GraphPad Software, San Diego, CA, USA). A $P < 0.05$ was considered statistically significant.

RESULTS

100 histopathologically confirmed cases of CAUB and 100 healthy controls were included. The details of baseline characteristics of CAUB patients and controls are given in Table 1.

On comparing the blood HM and TE levels, it was found that CAUB patients had a significantly higher mean value of As, ($P = 0.006$) Mn ($P < 0.0001$) and Pb ($P = 0.02$) levels than the control group. However, the blood concentration of Se was found to be significantly lower in CAUB patients ($P < 0.0001$) compared to the control group. The blood levels of Cu, Cd, and Hg levels were comparable between the two groups [Table 2 and Figure 1a, b]. 68% of CAUB patients had blood concentrations of HM and TE above the safe upper limits while 55% had blood concentrations of Mn higher than the permitted level. 68% patients had blood levels of Pb higher than the upper limit of the permitted level and 16% and 6% patients had higher Cu and Hg blood concentrations above the safe permitted levels, respectively.

Similarly, on comparing the urinary levels of HM and TE, we noticed significantly higher concentrations of As, Mn, and Pb in CAUB patients as compared to

Table 1: Clinicopathological characteristics (*n*=100)

Variable	CAUB cases	Control group	*P*†
Age (years)*	56.41±13.25	53.15±10.39	0.06
Sex			
Male	87	77	0.06
Female	13	23	
Smoking history			
Smoker	54	21	0.001
Nonsmoker	46	79	
Diet			
Vegetarian	50	38	0.12
Nonvegetarian	50	62	

†Independent *t*-test (continuous variables) or Fisher's exact test (categorical variables) was used to test for the significance. All values are expressed as *n* (%) except (*), which is expressed as mean±SD. CAUB=Carcinoma urinary bladder, SD=Standard deviation

Table 2: Elemental analysis in the blood between carcinoma urinary bladder patients and controls (*n*=100)

TE (μg/L)	Reference range	CAUB cases*	Control group*	*P*†	Patients having blood levels above permitted limit, *n* (%)	Controls having blood levels above permitted limit, *n* (%)
As	<62	0.80±0.09	0.45±0.08	**0.006**	0	0
Cu	1200–1400	1176±344.3	1140±523.6	0.21	16 (16)	12 (12)
Mn	4.2–16.5	38.62±46.86	11.06±8.25	**<0.0001**	55 (55)	25 (25)
Se	70–150	149.1±55.00	182.7±46.89	**<0.0001**	4 (4)	75 (75)
Cd	<5	0.25±0.65	0.29±0.55	0.56	1 (1)	0
Pb	<25	72.64±10.23	47.20±72.64	**0.02**	68 (68)	64 (64)
Hg	<10	1.67±5.12	0.23±0.76	0.33	6 (6)	0

*All values are expressed as mean±SD, †Independent *t*-test was used to compare between the two groups. $P<0.05$ signifies statistical significance and is shown in bold. CAUB=Carcinoma urinary bladder, SD=Standard deviation, TE=Trace elements, As=Arsenic, Cu=Copper, Mn=Manganese, Se=Selenium, Cd=Cadmium, Pb=Lead, Hg=Mercury

Table 3: Elemental analysis in the urine between carcinoma urinary bladder patients and controls

TE (μg/L)	Reference range	CAUB cases*	Control group*	*P*†	Patients having urine levels above permitted limit, *n* (%)	Controls having urine levels above permitted limit, *n* (%)
As	<35	12.44±10.42	5.30±6.27	**0.0001**	1 (1)	0
Cu	2.0–80	106.75±216.3	73.84±117.83	0.18	29 (29)	17 (17)
Mn	<1.4	2.64±4.34	1.40±3.60	**0.03**	34 (34)	17 (17)
Se	<200	37.40±47.20	39.49±30.80	0.72	2 (2)	0
Cd	<2.6	0.64±1.84	0.53±0.81	0.28	6 (6)	3 (3)
Pb	<80	12.54±4.1	2.26±0.42	**0.01**	4 (4)	0
Hg	<10	0.22±0.08	0.07±0.03	0.07	0	0

*All values are expressed as mean±SD, †Independent *t*-test was used to compare between the two groups. $P<0.05$ signifies statistical significance and shown in bold. CAUB=Carcinoma urinary bladder, SD=Standard deviation, TE=Trace elements, As=Arsenic, Cu=Copper, Mn=Manganese, Se=Selenium, Cd=Cadmium, Pb=Lead, Hg=Mercury

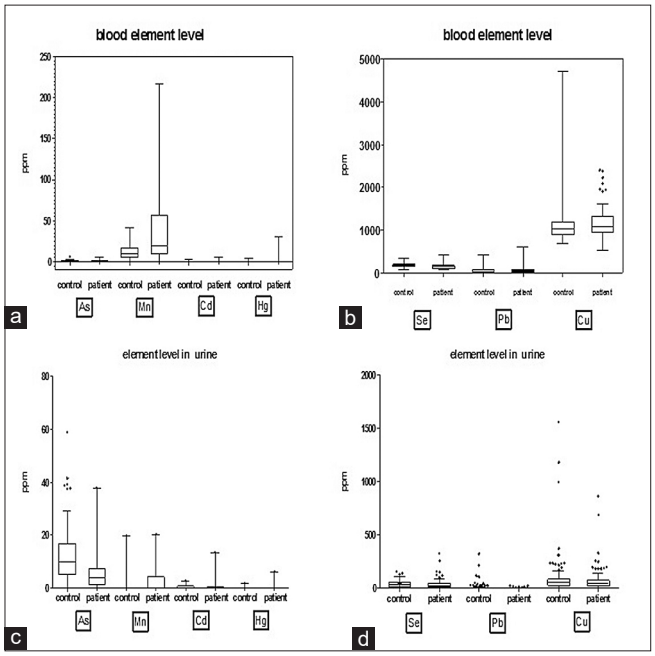

Figure 1: Comparison of blood (a and b) and urinary (c and d) levels of trace elements. TE = Trace elements, As = Arsenic, Cu = Copper, Mn = Manganese, Se = Selenium, Cd = Cadmium, Pb = Lead, Hg = Mercury

the controls. However, the urinary levels of Cu, Cd, and Hg did not differ significantly between the two groups [Table 3 and Figure 1c, d]. Further, 59% of CAUB cases had their urinary levels of HM and TE higher than the permitted level. Of the 100 controls, 34 (34%) and 29 (29%) patients had Mn and Cu concentrations higher than the permitted level in the urine, respectively.

On subgroup analysis, the blood and urinary levels of heavy metals and trace elements (HMTE) were comparable between low- and high-grade CAUB patients. On stratifying CAUB cases into noninvasive papillary (Ta) and invasive papillary or more (≥T1), we found that the blood Cu levels were significantly higher in patients with Ta stage than ≥T1 stage (1305 ± 497.7 vs. 1106 ± 194.3 μg/L, $P = 0.005$). There was no difference in other blood HMTE levels among the sub-groups. Similarly, the urinary concentration of Cu was comparable between Ta and ≥T1 stages [Supplementary Tables 1 and 2].

In CAUB cases, we observed a significantly lower serum GSH-Px (113.5 ± 44.75 vs. 163.9 ± 120.5, $P = 0.0002$) and SOD levels (11.35 ± 5.643 vs. 13.75 ± 3.999, $P = 0.008$) and a higher serum LPO levels (15.52 ± 14.78 vs. 11.18 ± 11.28, $P = 0.02$), compared to the control group. The comparison of serum antioxidant enzymes and LPO between the two groups is shown in Table 4.

DISCUSSION

HM and TE exist naturally in the environment and human exposure is inevitable. Various sources of HM and TE exposure include air, water, food, and occupation. Being a modifiable risk factor for various human illnesses, including cancer, HM and TE has been an important topic of research. Hence, epidemiological studies evaluating the association of HM and TE and various cancer development received considerable attention.[10] In the past, few studies have studied the role of HM and TE in CAUB, yet

Table 4: Serum antioxidant enzymes and lipid peroxidation assays

Variables	CAUB patients*	Controls*	*P*†
GSH-Px	113.5±44.75	163.9±120.5	**0.0002**
SOD	11.35±5.643	13.75±3.999	**0.0008**
LPO	15.52±14.78	11.18±11.28	**0.02**

*All values are expressed as mean±SD, †Independent *t*-test was used to compare between the two groups. $P<0.05$ signifies statistical significance and shown in bold. CAUB=Carcinoma urinary bladder, GSH-Px=Glutathione peroxidase, SOD=Superoxide dismutase, LPO=Lipid peroxidation, SD=Standard deviation

with heterogeneous results. These studies differed in samples used to measure the HM and TE and their association with CAUB. After exposure to HM and TE, the blood concentration of such elements increases initially, and hence measuring the blood level is a better guide to acute exposure. However, it is with continuous exposure the concentration of HM and TE increases in the urine. This may be due to saturation of TE binding sites, concentrations reaching above-permitted levels and/or renal-tubular leak. Hence, measuring both whole blood and urine concentrations are therefore required to know acute and chronic exposure, respectively.[9] In the present study, we analyzed the levels of HM and TE in the blood and urine of CAUB and controls, along with the comparison of redox status between the two groups.

Among studies evaluating the blood levels of HM and TE, a Belgian case-control study showed that the mean blood levels of Cd were higher in the CAUB cases (1.1 mg/L vs. 0.7 mg/L) than in the control group.[11] However, Gecit *et al*. showed that serum levels of Cd, Ni, and Co were increased while the levels of Mn and Zn were decreased in CAUB patients.[3] In the present study, we noticed higher blood levels of As, Mn, and Pb in CAUB patients than in controls. The varied differences in the levels of HM and TE in the above studies could be due to geographical differences in the study population. However, the differences in HM/TE levels in our study from the previous studies can be explained by doing further extensive studies taking into account geographical location and other confounding factors. Selenium is an important TE in the body with anti-oxidant properties. Inconsistent with prior studies on Selenium, blood Se levels were significantly lower in CAUB patients.[12]

Only a few studies evaluated the role of urinary levels of HM and TE in CAUB. In a pilot study, Malczyk *et al*. investigated the urinary Pb concentration in 24 biopsies-proven CAUB patients and found that urinary Pb levels were above the permitted level in 40% of cases.[13] The same group investigated urinary Cd in 10 CAUB patients and showed that 60% of patients had increased Cd levels above the permitted level.[14] Only two case-control studies evaluated urine levels of HM and TE in CAUB. In a study from Taiwan, Lin *et al*. noticed higher urinary levels of Zn and Se; and a lower As, Cu, and Pb levels in CAUB cases than controls.[4] Unlike previous studies, Guo *et al*. analyzed HM and TE in both serum and urine samples of CAUB and control groups. They found that serum Cu and urinary Zn levels were higher in CAUB cases. Of note, lower serum Ca and higher urinary Ca concentrations were found in the CAUB group.[15] It has been shown that measuring whole blood and urine concentrations would provide better insights into acute and chronic exposures of HM and TE, respectively.[9] In the present study, we found higher concentrations of As, Mn, and Pb both in blood (acute exposure) and urine (chronic exposure) of CAUB cases as compared to the control group. These findings signify long-standing exposure to HM and TE exposure in these patients. Supporting this, a few CAUB cases, as shown in Tables 2 and 3, had blood and urinary concentrations of these HM and TE above the permitted level. While there was no difference in the blood and urine levels of other HM and TE in terms of tumor invasiveness and grading, interestingly, the blood Cu levels were elevated in Ta as compared to ≥T1 stage.

Pertaining to tissue evaluation of HM and TE in CAUB, Abdel-Gawad *et al*. found high concentrations of Cd, Pb, Cr, Ni, and Zn in the tumor tissues.[10] They also showed that concentrations of As were high in the adjacent noncancerous tissues of radical cystectomy specimens and suggested their pathogenic role of these HM and TE in CAUB. Since this was our maiden attempt at HM and TE in CAUB, we only analyzed blood and urinary concentrations to evaluate the role of HM and TE in CAUB.

An imbalance in the HM and TE concentrations may lead to oxidative damage in the biological system as these TEs are an integral part of the enzyme's structure responsible for antioxidant protection. Hence, both increase and decrease of HM and TE ion content may impact the redox status.[3,16] While analyzing the antioxidant and LPO assays in our study, we found significantly lower GSH-Px and SOD levels in the serum of CAUB cases as compared with the control group. Further, a higher serum LPO concentration was also noted in the study group. The lower antioxidant enzyme levels and a higher LPO suggest the prevailing imbalance in the oxidative status in the CAUB group.

Overall, several implications are suggested by the present analyses. Blood and urinary concentrations of As, Mn and Pb are higher in the CAUB group suggesting the acute and chronic exposure of these HM and TE in CAUB cases. Further, 68% and 59% had their blood and urinary concentrations, respectively, above their permitted level, suggesting that these HM and TE might be risk factor for CAUB development. Selenium, the TE with antioxidant

properties, was significantly lower in the blood of CAUB cases. Finally, the study findings of lower antioxidant enzymes with concomitant higher LPO assays in the serum substantiate oxidative damage in CAUB cases than controls.

The current study suffers from a few limitations. We did not analyze an extensive panel of HM and TE. Only a few confounding factors were considered at baseline like smoking and dietary habits. We did not consider other factors like environmental exposures that include soil and water levels of HM, geographical differences, assessment of food source for HM and TE, occupational exposure, ayurvedic or homeopathic or alternative medicines usage, tobacco chewing, stratification of pack years of smoking. Similarly, we did not consider other pathological diseases, such as neurological diseases that can affect redox status while evaluating the antioxidant enzymes and LPO assays. We did not assay HM and TE levels in malignant and adjacent nonmalignant tissues to establish the role of tissue concentrations in CAUB. However, the present study is our initial attempt at HM and TE in CAUB and hence we tried to establish the role of HM and TE in blood and urine samples alone. Since the study established significant findings with respect to HM and TE and redox status, as an extension of this study, we would be analyzing HM and TE levels and genetic/epigenetic changes in tumor and adjacent tissues taking into consideration other confounding factors affecting HM and TE concentration such as geographical habitat, occupational exposure, and detailed dietary habits including seafood consumptions.

Finally, though HM and TE concentration in various biological samples may signify their potential role in cancer induction and progression, it is the genetic and epigenetic changes caused by HM and TE that establish the cause-effect relationship in CAUB cases. Hence, future studies are warranted to establish the causal relationship between HM and TE imbalance in terms of the molecular mechanism of carcinogenesis.

CONCLUSIONS

Significantly higher concentrations of As, Mn, and Pb noted in the blood and urine of CAUB patients, together with lower blood Se levels compared to the control group, may suggest their role in the pathogenesis of CAUB. Further, downregulated GSH-Px and SOD levels with increased LPO levels in the serum of CAUB cases imply oxidative stress in these patients.

REFERENCES

1. Kim HS, Kim YJ, Seo YR. An overview of carcinogenic heavy metal: Molecular toxicity mechanism and prevention. J Cancer Prev 2015;20:232-40.
2. Klaunig JE, Kamendulis LM, Hocevar BA. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 2010;38:96-109.
3. Gecit I, Kavak S, Demir H, Gunes M, Pirincci N, Cetin C, *et al*. Serum trace element levels in patients with bladder cancer. Asian Pac J Cancer Prev 2011;12:3409-13.
4. Lin CN, Wang LH, Shen KH. Determining urinary trace elements (Cu, Zn, Pb, As, and Se) in patients with bladder cancer. J Clin Lab Anal 2009;23:192-5.
5. Singh SP, Kaur S, Singh D. Food toxicology – past, present, and the future (the Indian perspective). In: Gupta RK, Dudeja, Singh Minhas, editors. Food sadety in the 21st Century. San Diego: Elsevier; 2017. p.91-110. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012801773900008X. [Last accessed on 2023 May 27].
6. Mir IA, SreePrabash M, Sridhar V, Maruthi KV. Distribution, sources and toxicity of heavy metals in surface sediments of North Western Karnataka, South India. Sci Rep 2022;12:15782.
7. Mawari G, Kumar N, Sarkar S, Frank AL, Daga MK, Singh MM, *et al*. Human health risk assessment due to heavy metals in ground and surface water and association of diseases with drinking water sources: A study from Maharashtra, India. Environ Health Insights 2022;16:1-11.
8. Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M. Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium, and arsenic. Front Pharmacol 2021;12:643972.
9. Panaiyadiyan S, Quadri JA, Nayak B, Pandit S, Singh P, Seth A, *et al*. Association of heavy metals and trace elements in renal cell carcinoma: A case-controlled study. Urol Oncol 2022;40:111.e11-111.e18.
10. Abdel-Gawad M, Elsobky E, Shalaby MM, Abd-Elhameed M, Abdel-Rahim M, Ali-El-Dein B. Quantitative evaluation of heavy metals and trace elements in the urinary bladder: Comparison between cancerous, adjacent non-cancerous and normal cadaveric tissue. Biol Trace Elem Res 2016;174:280-6.
11. Kellen E, Zeegers MP, Hond ED, Buntinx F. Blood cadmium may be associated with bladder carcinogenesis: The Belgian case-control study on bladder cancer. Cancer Detect Prev 2007;31:77-82.
12. Amaral AF, Cantor KP, Silverman DT, Malats N. Selenium and bladder cancer risk: A meta-analysis. Cancer Epidemiol Biomarkers Prev 2010;19:2407-15.
13. Malczyk E, Darewicz B, Pawlak D, Darewicz J, Buczko W. Investigations of urinary lead concentration in patients with urinary bladder carcinoma. Int Urol Nephrol 1999;31:661-3.
14. Darewicz G, Malczyk E, Darewicz J. Investigations of urinary cadmium content in patients with urinary bladder carcinoma. Int Urol Nephrol 1998;30:137-9.
15. Guo KF, Zhang Z, Wang JY, Gao SL, Liu J, Zhan B. Variation of urinary and serum trace elements (Ca, Zn, Cu, Se) in bladder carcinoma in China. Asian Pac J Cancer Prev 2012;13:2057-61.
16. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr J 2016;15:71.

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Supplementary Table 1: Comparison of elemental analysis in the blood of carcinoma urinary bladder patients

TE	Low-grade (*n*=38) versus high-grade (*n*=62)*	*P*†	Ta stage (*n*=35) versus ≥ T1 stage (*n*=65)*	*P*†
As	0.64±0.66 versus 0.89±1.11	0.19	0.85±0.84 versus 0.77±1.04	0.73
Cu	1260±408.8 versus 1124±289.7	0.06	1305±497.7 versus 1106±1 94.3	**0.005**
Mn	32.32±40.94 versus 42.48±50.07	0.29	39.6±53.12 versus 38.09±43.5	0.88
Se	156.5±52.98 versus 144.5±56.13	0.29	156.3±59.19 versus 145.1±52.6	0.33
Cd	0.19±0.41 versus 0.28±0.76	0.53	0.21±0.45 versus 0.27±0.73	0.73
Pb	68.14±89.41 versus 75.4±110.1	0.73	85.1±100 versus 65.9±103	0.37
Hg	2.58±6.12 versus 1.11±4.35	0.16	0.79±1.6 versus 2.14±6.19	0.21

*All values are expressed as mean±SD, †Independent *t*-test was used to compare between the two groups. $P<0.05$ signifies statistical significance and is shown in bold. TE=Trace elements, As=Arsenic, Cu=Copper, Mn=Manganese, Se=Selenium, Cd=Cadmium, Pb=Lead, Hg=Mercury, SD=Standard deviation

Supplementary Table 2: Comparison of elemental analysis in the urine of carcinoma urinary bladder patients

TE (μg/L)	Low-grade (*n*=38) versus high-grade (*n*=62)*	*P*†	Ta stage (*n*=35) versus ≥ T1 stage (*n*=65)*	*P*†
As	12.62±9.98 versus 12.33±10.77	0.89	12.23±9.47 versus 12.56±10.97	0.88
Cu	76.86±78.36 versus 125.1±267.1	0.28	112.6±204.4 versus 103.6±224	0.84
Mn	2.61±4.54 versus 2.65±4.24	0.96	2.2±4.3 versus 2.8±4.3	0.51
Se	33.18±35.72 versus 40.3±53.15	0.48	35.06±36.62 versus 38.7±52.25	0.71
Cd	0.93±2.53 versus 0.46±1.23	0.23	0.99±2.6 versus 0.44±1.23	0.16
Pb	9.83±21.7 versus 14.19±50.11	0.61	10.85±22.09 versus 13.45±49.12	0.76
Hg	0.29±1.04 versus 0.18±0.68	0.51	0.39±1.15 versus 0.13±0.58	0.13

*All values are expressed as mean±SD, †Independent *t*-test was used to compare between the two groups. $P<0.05$ signifies statistical significance and is shown in bold. TE=Trace elements, As=Arsenic, Cu=Copper, Mn=Manganese, Se=Selenium, Cd=Cadmium, Pb=Lead, Hg=Mercury, SD=Standard deviation