MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Investigations on the relationship between heavy metal toxicity and interstitial cystitis / bladder pain syndrome (IC/BPS)

Maha B. Bedier[2], Mohamed M. El Defrawy[1], Ahmed S. El-Hefnawy[2], Shereen E. Salem[1], Safaa A. Derbala[2]

[1] Chemistry Department, Faculty of Science, Mansoura University, Egypt.

[2]Urology and nephrology center, Mansoura University, Egypt.

**** Correspondence to:** mahabedier230@yahoo.com, 01001625970)*

Received: 6/4/2024
Accepted: 28/05/2024

Abstract: The illness known as interstitial cystitis/bladder pain syndrome (IC/BPS) is characterized by an overly sensitive bladder; usual causes are still unknown. The purpose of this study was to look into the association between the amounts of heavy metals Pb and Cd) in IC/BPS persons' blood and urine. Two groups of seventy Egyptian women were created: those with an IC diagnosis and healthy controls. The findings demonstrated that, in comparison to the control group, the blood and urine of IC patients had considerably higher amounts of Cd and Pb. Patients with IC were also found to have high cholesterol level. Even though they were still within in the normal range, IC patients had low levels of albumin and total protein. Urinary proteins did not substantially correlate with heavy metal levels in the blood or urine, while the protein / creatine ratio was significantly higher in IC patients than in the control group. According to this study, heavy metals may directly increase the risk of IC.

keywords: Interstitial cystitis (IC), Cd, Pb, Ca, Albumin, Cholesterol

1.Introduction

Bladder pain syndrome, formerly known as interstitial cystitis (ISC/BP), is a pelvic condition that affects or appears to affect the bladder. It was previously known as interstitial cystitis. This illness is characterized by lower urinary tract symptoms and chronic inflammation that cannot be attributed to an infection or any other recognized cause. The illness does not usually cause incontinence as a symptom. Because it is still considered an exclusion diagnosis, the illness is frequently mislabeled or identified too late, especially in males, as chronic pelvic pain syndrome (CP/CPPS) or chronic prostatitis (1). Patients frequently report experiencing severe need to urinate (urgency) together with pain in the bladder region (suprapubic). The sensation is exacerbated by filling the bladder, and it is frequently alleviated by increasing the frequency of urination. This could happen throughout the day or at night (nocturia). Other symptoms include dysuria, which is pain or burning when passing pee, and dyspareunia, which is discomfort during sexual activity. The patient's emotional, psychological, and social well-being as well as their quality of life is significantly impacted by these persistent symptoms (2).

ISC/BP mainly affects female patients with ratio of about nine female to one male. The prevalence for men is 8 to 41/100,000, while it ranges from 52 to 500/100,000 for women. The diagnosis of IC/BPS is mostly hampered by inadequate biomarkers, requiring extensive clinical examinations to rule out other "confusable" diseases (3) & (4).

Heavy metals come from a variety of sources, such as mining, agriculture, and industry. In the agricultural sector, fertilization, pesticides, livestock dung, and wastewater are all considered sources. The risk of heavy metal contamination in the environment has recently been rapidly increasing and causing chaos, especially in the agricultural economy. This is due to the accumulation of heavy metals in the soil and plant uptake (5).

Due to its ability to enter the body by natural or human activity and accumulate there, heavy metal contamination in food poses a serious risk to human health. While most harmful compounds are broken down either before or after being ingested, some persistent heavy metals, like lead in bones and copper in kidneys, stay in food and are permanently attached to bodily organs (6).

The urinary system's functions include eliminating heavy metals, poisons, and toxins from the blood, maintaining electrolyte balance, eliminating extra fluid, producing and transferring urine to the bladder. Due to this process, the kidneys and bladder are highly correlated with these heavy metals and toxins, which can result in a number of disorders in these two vital organs (7).

The body needs a variety of metals to stay in a state of balance. But it's important to distinguish between compounds that, although required in small amounts by living organisms, become poisonous at larger concentrations and those that are simply labeled as mandatory poisons without any particular metabolic role. Cadmium (Cd) and lead (Pb) are part of the latter group (8).

The World Health Organization (WHO) has designated ten substances as being especially dangerous to human health, of which are Cd and Pb (9). In addition, USA Agency for Toxic compounds and Disease Registry (ATSDR) ranked Pb in second position and Cd in seventh place on their priority list of hazardous (10).Despite their natural distribution, the smelting, battery, ceramic, and pigment industries raise the environmental concentrations of these substances (11).

In rats, lead or cadmium accumulates in the bladder as a result of subacute toxicity is linked with histopathological damage to the bladder. Urinary bladder function is disrupted by lead and cadmium because they interfere with the detrusor muscle's ability to contract and relax (12).

To the best of our knowledge, this study is the first to look into the connection between those who have interstitial cystitis and concentrations of heavy metals like Cd and Pb in urine and blood. The goal of the study is to offer new information on the aetiology of interstitial cystitis that may be useful in the diagnosis of the condition.

2. Materials and methods

(Times New Rom ,Font 12,single space)

2.1. Materials

In this study, 70 Egyptian women were split into two groups based on similarity in age, height, weight, and body mass index (BMI).Group [1] included 20 healthy women who have normal controls. Group [2] included 50 women with interstitial cystitis diagnoses.

❖ **Patients' ethics declaration**

The Mansoura University, Faculty of Medicine's Institutional Review Board (IRB) gave its approval to this study (Ref. 20-04-882).

2.2. Methods

2.2.1. Collecting of samples

Two tubes were filled with five milliliters of blood that were drawn from the peripheral venous system. Heavy metals including lead and cadmium as well as blood constituents like calcium, inorganic phosphorus, creatinine, uric acid, cholesterol and albumin were tested in the first tube. The complete blood picture (CBC) was measured using ethylene diamine tetra-acetic acid (EDTA), which was contained in the other tube.

Urine samples were collected in 50ml polypropylene tubes and centrifuged for 10 minutes at 1500 rpm to remove any debris. 5ml urine was used to determined creatinine and urinary protein. The remaining urine samples were kept at -20°C in polypropylene tubes that had been acid-washed for Cd and Pb measurement.

Using a Beckman Coulter (AU480) fully automated system, all biochemical parameters, including creatinine, calcium, inorganic phosphorus, total protein, albumin, and cholesterol were automatically measured at baseline in all experimental individuals in compliance with standard protocols according to (13), (14), (15), (16), (17) and (18).

The System XP-300TM Automated Hematology Analyzer was used to measure the CBC.

2.2.2. Examination of Cadmium and lead

For the Cd and Pb tests, venous blood samples from each participant were taken, and the samples were kept in tubes at -20 °C. Using the GBC Scientific Equipment Atomic Absorption Spectrophotometer "SENSAA Model" at the Atomic Absorption Unit, Faculty of Science, Mansoura University serum samples were tested for Cd and Pb at wavelengths of 217 nm and 228.8 nm, respectively.

Urine samples containing Cd and Pb were analyzed by pouring 5 ml of urine into a 15 ml Pyrex tube and heating it to 80 ºC for five hours. Following cooling, the tubes were shook, closed, and then set again at (80–90) ºC for 30 minutes. Five milliliters of $KMnO_4$ was then added. After cooling the digest, a NH_2OH supersaturated solution was added.

2.2.3. Experimental materials

Lead nitrate salt Pb $(NO_3)_2$was used to prepare (100 $mg.L^{-1}$) of lead (II) stock solution and cadmium chloride salt$CdCl_2.3H_2O$ was used to prepare (100 $mg.L^{-1}$) of cadmium (II) stock solution. To modify pH, standard aquatic solutions including HCl and NaOH were used. By weighing the required amount in distilled water, tock solutions were made. The anions were used as their potassium or sodium salts, and the metal salts as their chlorides. All of the functional solutions were created by diluting using deionized water.

Technique for ascertaining as stated in **Table.1**, GBC Scientific Equipment "SENSAA Model" atomic absorption spectrophotometer, (computerized AAS) with air acetylene flame was used to assess Cd and Pb in the blood and urine samples under ideal instrumental circumstances.

Table (1): Optimum instrumental conditions of Cd and Pb.

Characteristic	Wavelength (nm)	Working calibratingrange (ppm)	Sensitivity (µg/ml)
Cd	228.8	0.2-1.8	0.009
Pb	217	2.5-10	0.06

The following equations were used to calculate the heavy metals concentrations.

For cadmium concentration = Abs/(0.2127+ -0.1413*Abs)

For lead concentration =Abs/ (0.0209+0.0129*Abs)

In order to balance the impact of the urine flow rate on the excretion rate, the urinary creatinine was adjusted to compute the concentrations of urinary Cd and Pb.

2.2.4. Statistical analysis

The statistical package for the social sciences, Windows version 17USA (SPSS PC+ version 17 software) was used for data analysis. For variables with a normal distribution, the formula was mean +/- SD. To compare groups, the sample T test was utilized. A P-value of less than 0.05 was considered significant.

3. Results and Discussion

(Times New Rom ,Font 12,single space)

The study groups' demographic information is listed in **Table.2**. Age, height, weight, and BMI did not significantly differ across the study groups (P>0.05).In addition, the subjects in studied groups didn't suffer from diabetics or hypertension.

Table (2): The demographic data of the studied groups

Characteristic	Group 1 (20)	Group2 (50)	P value
Age (years)	38.3± 7.78	36.94± 9.97	0.587
Weight (Kg)	72±10.99	78.76±15.92	0.087
Height (cm)	163.0±8.33	163.02±7.83	0.992
BMI (Kgm^2)	27.58±6.31	29.93±7.24	0.208
Diabetic			1
Yes	0	0	
No	20	50	
Hypertension			1
Yes	0	0	
No	20	50	

Table.3 shows the Pb and Cd in blood and urine concentrations for Group 1 (control group) and Group 2 (IC group). The IC group's blood Pb and Cd concentrations were considerably greater than the control group's (p = 0.004 and 0.002, respectively). **Table.3** displays the urine Pb and Cd values from the control group (Group 1) and IC group (Group 2). The IC group had significantly greater urinary concentrations of Cd / creat and Pb / creat (p = 0.028 and 0.001, respectively) than

the control group. Lead in the blood and urine have a direct association (r=0.523 & P=0.001), and cadmium in the blood and urine have an inverse correlation (r=-0.386 & P=0.006).

Table (3): blood and urine heavy metals in studied groups

Parameter	Group 1 (20)	Group2 (50)	P value
Serum Cd (ppm)	0.03± 0.001	1.713± 0.33	0.002
Serum Pb (ppm)	0.012±0.001	0.014±0.002	0.001
Urinary Cd/ creatinine ratio	0.00033± 0.00006	0.00053± 0.00004	0.028
Urinary Pb/ creatinine ratio	0.0039±0.0007	0.014±0.0093	0.001

Table.4 displays the biomarkers for the study groups (2). The study groups did not exhibit a significant difference in serum calcium and creatinine (P = 0.138 & 130). Phosphate and cholesterol levels in the IC serum were significantly higher than in the control group (P= 0.001 & 0.036). The albumin and total protein levels in IC patients were considerably lower (P= 0.001& 0.005) than in the control group. Furthermore, IC patients had a significantly higher urine protein / creatinine ratio than the control group (P=0.001).

Table (4): The biomarkers of the studied groups

Biomarker	Group 1 (20)	Group2 (50)	P value
Creatinine mg/dl	0.72± 0.1	0.66± 0.08	0.138
Cholesterol mg/dl	182±15.1	215.5±34.7	0.001
Total protein mg/dl	7.75±0.34	7.34±0.37	0.005
Albumin mg/dl	4.43±0.17	4.16±0.16	0.001
Ca mg/dl	9.98±0.4	9.75±0.43	0.130
Phosphorus mg/dl	3.2±0.43	3.63±0.6	0.036
Protein/creatinine ratio	0.067±0.035	0.23±0.016	0.001

The levels of hematological parameters were shown in **Fig.1**. Hemoglobin (Hgb) and red blood cells (RBCs) in patients with IC were considerably ($P \leq 0.05$) lower than in the healthy control group. However, compared to those in the healthy control group, IC patients had significantly greater white blood cell (WBC) counts ($P \leq 0.01$).

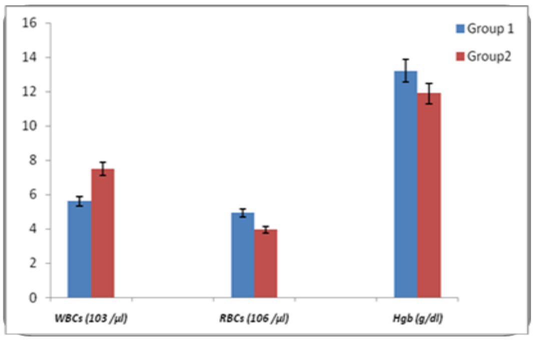
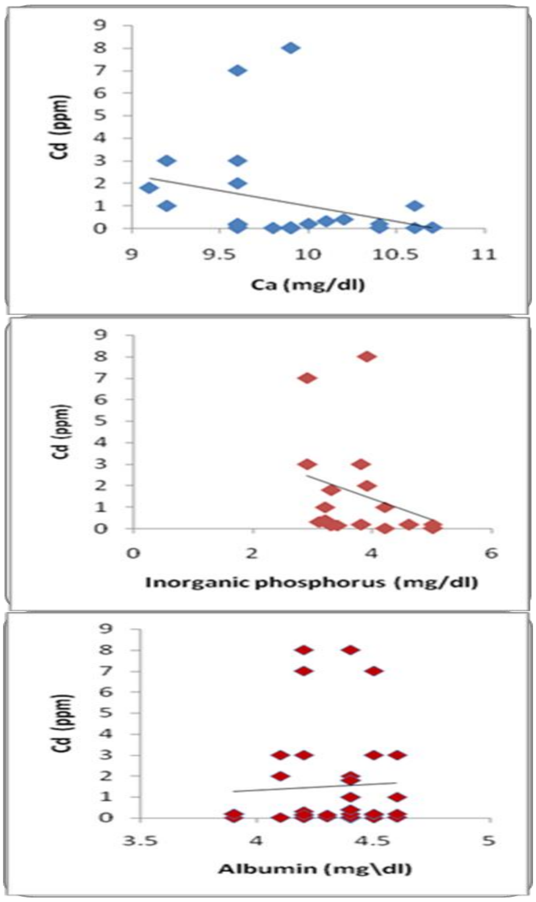


Figure (1): Hematological parameters in studied groups.

Fig.2 (1-5) displayed the correlation between serum IC group albumin and levels of Cd (serum and urine) and between Ca and inorganic phosphorus (P). There was inverse correlation between Cd and Ca (r= -0.480 & P=0.04), Cd and inorganic phosphorus (r=-793 &P=0.006). It was demonstrated a positive connection between albumin and blood levels of Cd (r= 0.470 & P=0.001), urinary Cd/creatinine ratio(r= 0.392 & P=0.005), and between urinary Cd/creatinine ratio and Pb/creatinine ratio (r=0.914 & P=0.001). There was no correlation between protein in the urine and urinary heavy metals in IC patients.



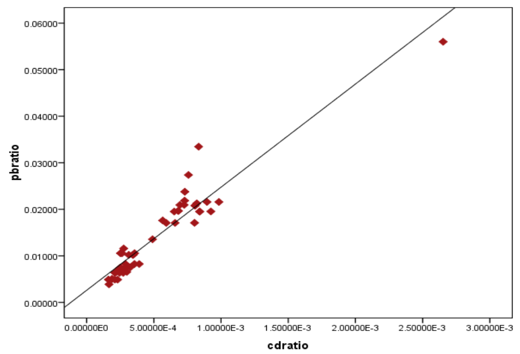


Fig (2): The correlation between heavy metals and other parameters.

In this study, Receiver Operating Characteristic (ROC) analysis in IC/BPS patients showed that Pb had a sensitivity of 76% and specificity of 80% with an area under curve (AUC) of 0.884, while serum Cd may be a predictor with a sensitivity of 90% and specificity of 100% with an area under curve (AUC) of 0.942 (**Fig.3**). Pb and Cd may be valuable as IC/BPS diagnostic biomarkers, according to the ROC curve analysis.

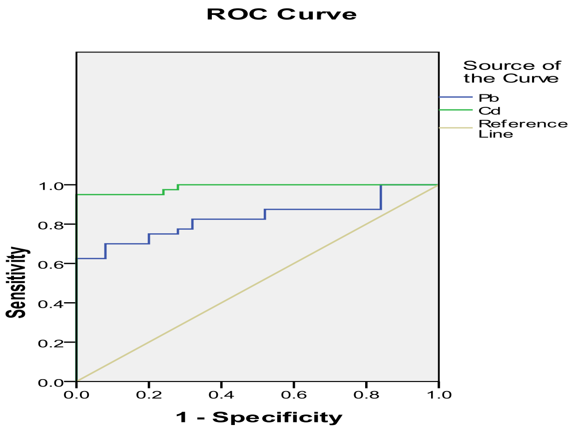


Fig (3): ROC curve for serum heavy metals (Cd &Pb levels (ppm) in IC/BPS patients

4. Discussion

The protective epithelial lining of the bladder, for example, may present with complications in patients with interstitial cystitis. Due to an epithelial leak, urine pollutants may irritate your bladder's wall. It is uncertain what specifically causes interstitial cystitis, however a number of factors might be involved. A multitude of theories exist about the cause of IC/BPS. They consist of the following: autoimmune, infections, food, environmental variables, elevated uroepithelial permeability, elevated mast cell activity and inflammatory response, and lack of glycosaminoglycan on the bladder's surface uroepithelial (19).

The human urinary system consists of the bladder, ureters, and kidneys. Because of the urinary system's duties also include eliminating excess fluid, creating urine, transporting it to the bladder, maintaining electrolyte balance, and eliminating chemicals, heavy metals, and poisons from the blood. Significant toxin and heavy metal contamination of the kidneys and bladder result from this process, which can lead to a number of ailments in these two essential organs. Thus, lowering exposure to heavy metals can lower the risk of developing kidney and bladder cancer as well as other illnesses involving this system (20). Because of their toxicity, protracted environmental half-lives, and capacity for bioaccumulation, heavy metals are well-known environmental contaminants. Although their natural sources are volcanic eruptions and the weathering of metal-containing rocks, mining and other industrial and agricultural operations are where they originated from humans (21).

This is the first study to demonstrate the connection between heavy metals (Pb and Cd) and interstitial cystitis/bladder pain syndrome (IC/BPS). Diagnosing Pb and Cd levels aids in the diagnosis of metal overexposure. Pb and Cd levels in the research patients (IC/BPS) were significantly greater than those in the control group ($P<0.001$ and 0.001, respectively). The higher levels in the study group did not exceed the requirements for Cd toxicity or the Pb toxicity limits set by the global communities. Adults' average blood lead levels can be as high as 0.1 ppm (22), while typical blood cadmium content is less than 0.005 ppm (23).

In the current investigation, urinary levels of Pb and Cd were shown to be higher in IC patients than in control volunteers. The protein/creatinine ratio in IC patients was significantly higher than in the control group. Urinary proteins and heavy metals (Pb and Cd) in blood or urine did not significantly correlate, according to statistical analysis. According to Chaumont *et al*., multivariate analysis revealed a strong link between lead, β (2)-microglobulin, and urinary retinol-binding protein and cadmium in urine, but not between the metals in blood (24).

Certain element ions like Cd and Pb were reported to be higher in CKD in earlier research

(25). Emam *et al*. reported that the blood of CKD patients had greater amounts of Cd and Pb than that of the control group (26). Because CKD patients had greater blood levels of Cd and Pb than the control group, the study's findings were mainly verified. Furthermore, there is a correlation between elevated levels of Cd and Pb and tumors including bladder cancer (27) and urotheilai carcinogenesis (28).

In contrast to the healthy control people in this study, IC/BPS patients had higher blood cholesterol. Cadmium exposure may block the enzyme lipoprotein lipase, which is involved in the metabolism of triglycerides and free fatty acids (29). This could result in an increase in blood levels of total cholesterol and triglycerides.

The albumin levels of IC patients were significantly lower in the current study ($P<0.05$) than those of the healthy control group. Albumin is an antioxidant and necessary plasma protein that is only produced by the liver. When the liver is injured, albumin is oxidized, changing its molecular structure, conformation and resulting in a decrease in the serum concentration of the protein (30). Lead and cadmium injuries occur almost through the same route (31).

The results of the study indicate that blood phosphorus levels in the groups of IC patients increased noticeably while remaining within normal ranges, however there was no discernible variation in blood calcium levels across the study groups. It was also demonstrated that there was a negative correlation between Pb and/or Cd and calcium and phosphorus. Variations in the serum concentrations of key elements could indicate possible pathways for the detrimental effects of cadmium and/or lead on human health.

Argade *et al.* state that lower levels of proteases in urine compared to plasma or less dilution of proteins discharged in urine may be the cause of the urinary rise of proteins associated with painful bladder syndrome/interstitial cystitis (32).

Patients with interstitial cystitis showed negative correlations between blood lead and cadmium and serum albumin, as well as between calcium and lead. Furthermore, there was a positive link found between albumin and the serum cadmium level and the urine Cd creatinine ratio. This study raises the possibility that CKD risk may directly correlate with the expression of heavy metals (Pb and Cd). Furthermore, this research can encourage the future development of a fresh preventative approach that focuses on the environment in order to avoid and manage interstitial cystitis.

According to ROC curve analysis, the diagnostic performance of heavy metals in blood showed 90% sensitivity and 100% specificity for Cd and 76% sensitivity and 80% specificity for Pb in IC.

5. Conclusion

Negative associations were seen between blood lead and calcium, as well as between blood lead and cadmium and serum albumin, in patients with interstitial cystitis. According to this study, chronic kidney disease (CKD) and the expression of heavy metals (Pb and Cd) may be closely related. Furthermore, this study might have sparked the creation of a fresh preventive plan that goes after the surroundings in the future to aid in controlling and avert interstitial cystitis. We recommend more broadly based research to accentuate the current findings and ensure that the possibility of being a future target for the genesis of the disease is preserved.

Conflicts of interest

The authors declare that they have no conflicts of interest.

6. References

1 Lim, Yizhe, Leslie, Stephen W. and O'Rourke, (2023) Seanan."Interstitial Cystitis. In: StatPearls [Internet]. Treasure Island (FL)", ,StatPearls.

2 Bogart L.M., Berry S.H. and Clemens; (2007) "Symptoms of interstitial cystitis, painful bladder syndrome and similar diseases in women": a systematic review., , *J. Urol.* , pp. **177(2)**:450-6.

3 Neuhaus J., Gonsior A. and Berndt-Paetz, M.; (2021) "Biomarkers in the Light of the Etiopathology of IC/BPS"., *Diagnostics (Basel)*, p. **11(12).**

4 Davis N. F., Brady C. M. and Creagh T.; (2014) "Interstitial cystitis/painful bladder syndrome: epidemiology, pathophysiology and evidence-based

treatment options"., *Eur J Obstet Gynecol Reprod Biol*, pp. **175**:30–37.

5 Rashid A., Schutte B.J, Ulery A., Deyholos M.K., Sanogo S., Lehnhoff E. A. and Beck L.; (2023) "Heavy Metal Contamination in Agricultural Soil: Environmental Pollutants Affecting Crop Health"., Agronomy, pp. **13(6)**, 1521.

6 Abdel-Rahman, Gomaa N.; (2022) "Heavy metals, definition, sources of food contamination, incidence, impacts and remediation A literature review with recent updates"., *Egyptian Journal of Chemistry*, pp. Vol. **65**, No. 1, pp. 419 - 437.

7 Khalaf E. M., Taherian M., Almalki S.G., Asban P. ,Kareem A. K. , Alhachami F. R., Almulla A. F., Romero-Parra R. M., Jawhar Z. H., Kiani F. , Manesh I. N. and Mohammadi M. J; (2023) "Relationship between exposure to heavy metals on the increased health risk and carcinogenicity of urinary tract (kidney and bladder)"., Rev Environ Health.

8 Furtak G., Kozłowski M., Kwiatkowski S. and Cymbaluk-Płoska A.; (2022), "The Role of Lead and Cadmium in Gynecological Malignancies". Antioxidants(Basel), pp. **11(12)**, 2468.

9 Geneva, Switzerland (2018) Concern. WHO Action Is Needed on Chemicals of Major Public Health Concern. (accessed on 26 September;Public Health Environmental. WHO. s.l. : WHO Action, (2010).

10 ATSDR ’s Substance Priority List. ATSDR, s.l. (2018).

11 Awadalla A., Mortada W. I., Abol-Enein H. and Shokeir A. A.;. (2020) "Correlation between blood levels of cadmium and lead and the expression of microRNA-21 in Egyptian bladder cancer patients, Heliyon", p. **6(12).**

12 Taha, S. S, Daabees T. T., Aly R. G. , and Senbel A. M ; (2022) , "Lead acetate versus cadmium sulfate in the modulation of main physiological pathways controlling detrusor muscle contractility in rat" *Saudi Pharm J*, pp. **30(3)**: 306–316.

13 Heinegard D. and Tiderstom G. ; (1973) "Determination of serum creatinine by a direct colometric method"., Clin. Chim. Acta, pp. **43**:305-310.

14 Michaylova V.; (1971), "Photometric determination of micro amounts of calcium witharsenazo III". Anal.Cem.Acta, pp. **53**:194-198.

15 Dryer R. L., Routh J. I.and McNAIR R. D. ; (1963) "Determination of serum inorganic phosphorus, stand. methods"., Clin.Chem, pp.**4**:191-195.

16 Fujita Y., Mori I. and Kitanor S.; (1983) "Color reaction between red molybdenum (VI) complex and protein"., Bunsaki Kagaku, pp. **32**:379-386.

17 Doumas B. T., Watson W. A. and Biggs H. G.; (1971) "Albumin standards and the measurement of serum albumin with bromocresol green" , Clin. Chim, pp. **23**:414-416.

18 Beale R. N. and Croft D.; (1962) "The determination of cholesterol in serum by persulphuric acid oxidation"., *J Clin Pathol*, pp**. 15(3)**: 221–227.

19 Aydogan T. B., Gurpinar O. , Eser O. K. , Mathyk B. A. and Erge A.; (2019),"A new look at the etiology of interstitial cystitis/bladder pain syndrome: extraordinary cultivations".*International Urology and Nephrology*, Vols. **51(11)**:1961-1967.

20 Khalaf, E. M., Taherian M. , Almalki S.G. , Asban P. , Kareem A. K. , Alhachami F. R. , Almulla A.F. , Romero-Parra R. M. , Jawhar Z. H. , Kiani F. , Manesh I. N. and Mohammadi M. J.; (2023) "Relationship between exposure to heavy metals on the increased health risk and carcinogenicity of urinary tract (kidney and bladder)". *Rev Environ Health*.

21 Ali H., Khan, E. and Ilahi I.; (2019), "Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation". *Journal of Chemistry*, Vol. **2019**.

22 Gunnar F., Nogawa, Koji , Nordberg and Monica Nordberg. "Handbook on the Toxicology of Metals" (Fourth Edition). (2015), Volume **II** pages:667-716

23 Center for Disease Control and Prevention.The National Institute for

Occupational Safety and Health (NIOSH).Adult Blood Lead Epidemiology and Surveillance (ABLES). (2016), Vols. **(55)**; 59–65.

24 Chaumont A., Nickmilder M. , Dumont X. , Lundh T., Skerfving S. and Bernard A. ; (2012) "Associations between proteins and heavy metals in urine at low environmental exposures: evidence of reverse causality". Toxicol Lett,Vols. **210(3)**:345-52.

25 El-Sherbeny S. S., Abd Alla A. M. and Massoud W.; (2006) "Copper, Zinc, Selenium, Cadmium and Lead Levels in plasma of Hemodialysis Patients". *The Egyptian Journal of Hospital Medicine*, Vols. **24**: 556 – 563.

26 Emam A., Hamed S., Elmowafy A., Awad M., Eldakroory S. and Elmansoury A.; (2020), "Relation between blood lead and cadmium levels and chronic kidney disease". Nephrology Dialysis Transplantation, Vols. **35 (S3)**;1063-1064.

27 Feki-Tounsi M., Olmedo P., Gil F., Khlifi R., Mhiri M. N., Rebai A. and Hamza-Chaffai A.; (2013), "Cadmium in blood of Tunisian men and risk of bladder cancer: interaction with arsenic exposure and smoking". *Envirn.Sci.Pollut.Control Ser*, Vols. **20(10)**:7204-13.

28 Chang C-H, Liu C-S, Liu H-J , Huang C-P, Huang C-Y, Hsu H-T , Liou S-H and Chung C-J; (2016), "Association between levels of urinary heavy metas and increase risk of urothelial carcinoma". *International Journal of Urology*, Vols. **23(3)**:233-9.

29 Nabilla V. A., Lestari I., Rahayuningsih C. K. and Christyaningsih J.; (2022) "Effectiveness of Moringa Leaf Extract (Moringa oleifera) on Cadmium and LDL (Low-Density Lipoprotein) Cholesterol Levels in Blood as Indicators of Atherosclerosis". *Journal Tecknokes*, Vols. **15(3)**:128-136.

30 Spanidis Y., Priftis A., Stagos D., Stravodimos G. A., Leonidas D. D. , Spandidos D. A., Tsatsakis A. M., and Kouretas D.; (2017) "Oxidation of human serum albumin exhibits inter-individual variability after an ultra-marathon mountain race". Exp Ther Med, Vols. **13(5)**:2382-2390.

31 Balali-Mood M., Naseri K., Tahergorabi Z., Khazdair M. R. and Sadeghi M. ; (2021) "Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic". Front Pharmacol, , Vol. **12.**

32. Argade S., Chermansky C. and Tyagi P.; (2016), "Biomarkers for Interstitial Cystitis/Painful Bladder Syndrome". Womens Health (Lond), Vols. **12(1)**: 87–90.