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Serum CA19-9, TLR4, Osteopontin, and Cadmium as Potential Biomarkers in Urolithiasis and Urinary Tract Infections: A Case-Control Study

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Abstract

Urolithiasis is a common urinary disorder frequently associated with recurrent stone formation and urinary tract infections (UTIs). This case–control study aimed to evaluate serum levels of carbohydrate antigen 19-9 (CA19-9), Toll-like receptor 4 (TLR4), osteopontin (OPN), and cadmium (Cd) in patients with urolithiasis and UTIs to explore their potential as biomarkers. A total of 160 participants were recruited: 58 with urolithiasis, 60 with UTIs, and 42 healthy controls. Serum CA19-9, TLR4, and OPN were measured using ELISA, while cadmium levels were determined by atomic absorption spectrophotometry. Results showed significantly elevated CA19-9 in urolithiasis patients (8.92 ± 0.68) compared with UTIs (4.89 ± 0.43) and controls (4.36 ± 0.23). TLR4 was markedly higher in UTI patients (1.74 ± 0.15) than in urolithiasis (0.94 ± 0.21) and controls (0.88 ± 0.14), while OPN was highest in controls (1.34 ± 0.12) and lowest in urolithiasis (0.93 ± 0.12). Cadmium concentrations were significantly elevated in urolithiasis patients (2.04 ± 0.22) compared with controls (0.88 ± 0.12). Correlation analysis revealed strong positive associations between TLR4 and cadmium and negative correlations of OPN with both inflammatory and toxic markers. These findings suggest that altered levels of CA19-9, TLR4, OPN, and cadmium may be associated with urolithiasis and UTIs. However, results should be interpreted cautiously due to the limited sample size, single-center design, and potential confounding factors such as diet, smoking, and environmental exposure. Larger multicenter studies are recommended to validate these observations and clarify underlying mechanisms.

Keywords: Urolithiasis**,** Toll-like receptor 4, Cadmium**,** Carbohydrate antigen 19-9.

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Introduction

Urolithiasis is one of the most common urological conditions, with a global prevalence ranging from 1% to 20%. Higher prevalence rates are observed in countries with a high standard of living. This disorder is associated with recurrent stone formation, significant morbidity, and substantial healthcare costs [1]. It is characterized by the presence of calculi in the urinary tract or kidneys, which may cause pain, obstruction, hematuria, and infection. Despite advances in surgical and medical management, recurrence rates remain high, reaching 70–81% in men and 47–60% in women [2]. Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide and frequently coexist with kidney stones. This coexistence creates a vicious cycle: obstruction and urinary stasis promote infection, while infection and inflammation can accelerate stone growth [3]. Acute UTIs can result in an abrupt decline in renal function, particularly when combined with urinary tract obstruction. Urolithiasis can also lead to secondary problems such obstruction or UTIs [4]. Urolithiasis patients frequently have concurrent UTIs and regardless of the chemical makeup of the stone, frequently have positive urine cultures before or after surgery [5].Urease-producing bacteria, such as *Escherichia coli* and *Proteus* species, alter urinary chemistry by hydrolyzing urea, leading to alkaline urine and favoring struvite and carbonate apatite stone formation [6]. Stone formation occurs in six steps including Urinary supersaturation, Crystallization, Crystal nucleation, Crystal aggregation [7]. Urinary stones are categorized into five major types; Calcium oxalate stones (calcium oxalate and calcium phosphate), Uric acid stone, Struvite stone, stone of cystine, Drug-Induced Stones [8]. Although imaging modalities and routine laboratory parameters assist in diagnosis, they provide limited insight into the molecular pathways driving stone formation and infection. The search for reliable **serum biomarkers** that capture the interplay between inflammation, infection, and toxic exposures is therefore of great clinical importance. Several candidates have been investigated individually. **Carbohydrate antigen 19-9 (CA19-9)**, while classically recognized as a tumor marker, is also elevated in benign obstructive urological conditions such as hydronephrosis, with levels falling after relief of obstruction [9,10]. **Toll-like receptor 4 (TLR4)** is a key mediator of innate immune activation in response to bacterial lipopolysaccharide and contributes to UTI-associated inflammation [11]. **Osteopontin (OPN)**, a multifunctional glycoprotein expressed in the kidney, inhibits nucleation, aggregation, and

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adhesion of crystals; reduced expression may predispose to stone formation [12]. **Cadmium (Cd),** is a toxic heavy metal with a biological half-life of 25–30 years, accumulates in the renal cortex, induces oxidative stress, and has been linked to increased risk of kidney stones in epidemiological studies [13,14]. This work aims to investigate the serum levels of CA19-9, TLR4, OPN, and cadmium in patients with urolithiasis and urinary tract infections compared with healthy controls, in order to evaluate their potential utility as predictors biomarkers.

Materials and Methods

This case-control study was that conducted at Al-Imam Al-Hassan Al-Mujtaba Teaching Hospital and Imam Hussein Medical City, between January to February 2025. A convenience sampling method was applied. A total of 160 participants were enrolled and categorized into three groups: Group I: 58 patients diagnosed with urolithiasis Group II: 60 patients with urinary tract infection (UTI). Group III (Control group): 42 apparently healthy individuals with no history of autoimmune or metabolic diseases. The participants age ranged between 20 and 70 years. In this study, the concentration of osteopontin (OPN) was determined using an Enzyme-Linked Immunosorbent Assay (ELISA) kit (BT LAB, China, CAT.NO:SL131Hu).and the concentration of toll like receptors 4 (TLR4) was determined using (ELISA) kit (BT LAB, China, CAT.NO:SL2269Hu) Carbohydrate antigen19-9 (CA19-9) concentration was determined also by (ELISA) kit (BT LAB, China, CAT.NO:SL0401Hu). Standards and samples were loaded into microplate wells, with one well left blank as a control. Plates were incubated at 37 °C, washed, HRP-conjugate added, incubated and washed again, followed by chromogen addition and color development for 15 min. The reaction was stopped, and absorbance read at 450 nm. All procedures were performed according to the manufacturer's instructions and samples were measured in duplicate to ensure accuracy concentration of cadmium parameters measurement was performed on blood serum by use (Atomic absorption spectrophotometer), model Buck Scientific - 210VGP Atomic Absorption Spectrophotometer. Serum samples were digested with nitric acid and hydrogen peroxide, diluted, filtered, and analyzed by atomic absorption spectrophotometry to determine heavy metal concentrations [15,16].

Inclusion criteria: Adults aged 20–70 years, diagnosed with urolithiasis (kidney, ureter, or bladder stones) or UTI, and willing to provide written informed consent.

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Exclusion criteria: Patients on dialysis, renal transplant recipients, individuals with known malignancy (e.g., prostate or renal cancer), pregnancy, chronic systemic diseases (e.g., diabetes, autoimmune disorders), recent antibiotic or immunosuppressive therapy (within the last 3 months), occupational exposure to heavy metals, and current smokers.

Ethical approval

This study was approved by the Ethical Committee at College of Science/University of Kerbalaa. All participants involved in this work were informed and agreement obtained orally from each one before the collection of the sample.

Statistical analysis

Data distribution was assessed using the Shapiro–Wilk test. Between-group comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons.

Results

The clinical characteristics including160; patient the age distribution of the study participants was assessed among three groups: patients with urolithiasis or kidney stone (n=58), patients with urinary tract infections(n=60), and healthy control (n= 42). (Figure 1) showed the mean age of the urolithiasis group was found to be 32.9 ± 2.45 years. For the UTI group the average age was recorded as 31.6 ± 3.56 years. In comparison, the control group showed a mean age of 29.8 ± 3.18 years. Statistical analysis revealed that there was no significant difference in age between the groups, as indicated by a p-value greater than 0.05.

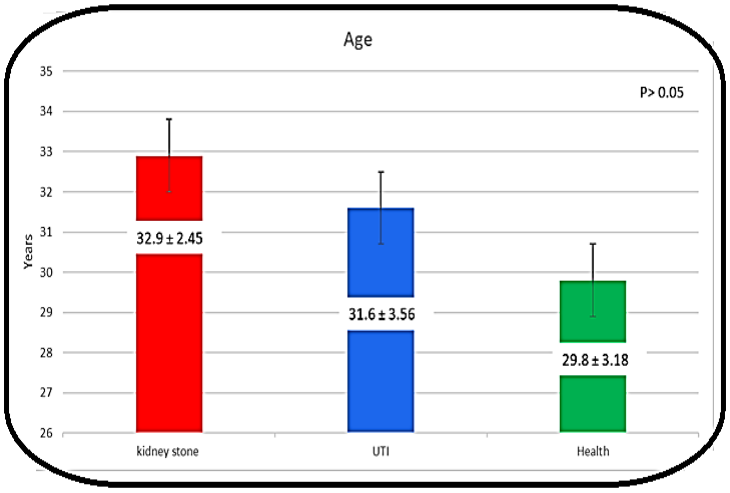

Figure 1. Distribution of participants according to ages.

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According to the statistical analysis in this study the serum CA19.9 level showed a progressive increase from controls (4.36 ± 0.23 ng/mL) to UTI patients (4.89 ± 0.43 ng/mL), reaching the highest in urolithiasis cases (8.92 ± 0.68 ng/mL); $p<0.001$ shown in (Figure 2) suggesting its possible link to urolithiasis and urinary tract infections**.**

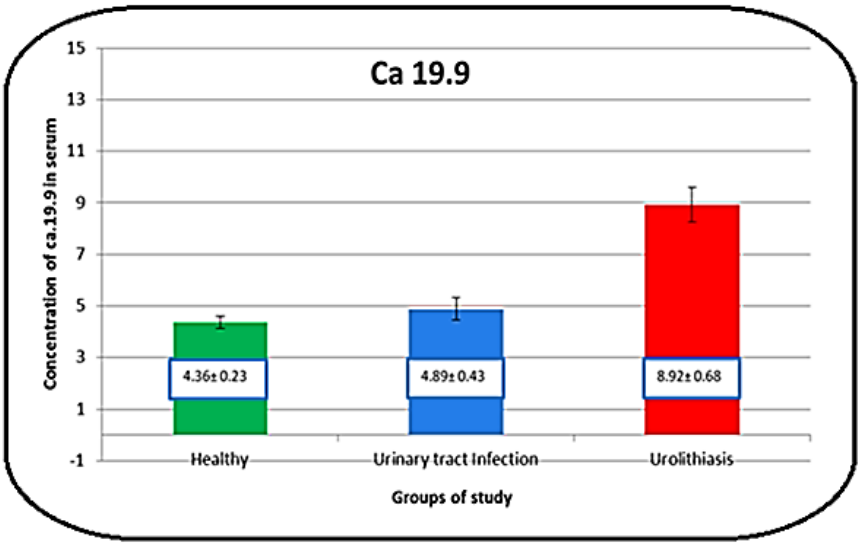

Figure 2. The concentration of Ca19.9 among participants.

The serum levels of Toll-Like Receptor 4 (TLR4) were evaluated across all study groups. A markedly elevated concentration of TLR4 was detected in group with urinary tract infections, with a mean value ±SD of 1.74 ± 0.152 ng/mL. In patients diagnosed with urolithiasis, the average TLR4 level was measured at 0.935 ± 0.21 ng/mL. In contrast, the control group exhibited the lowest TLR4 levels, with a mean of 0.88 ± 0.14 ng/mL, $p<0.001$ shown in (Figure 3). The results demonstrated a significant rise in TLR4 expression among patients with urolithiasis and UTIs compared to healthy individuals, indicating a possible involvement of TLR4 in the inflammatory responses associated with these conditions.

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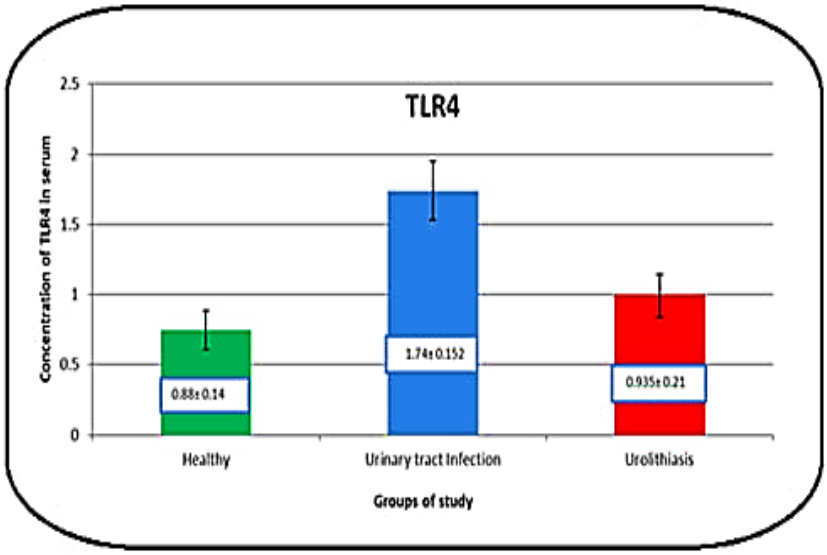

Figure 3. The concentration of TLR4 among participant.

Based on Figure 4, the Osteopontin levels were highest in controls (1.34 ± 0.118 ng/mL), lower in UTI patients (1.03 ± 0.37 ng/mL) $p<0.05$, and lowest in urolithiasis cases (0.934 ± 0.121 ng/mL), suggesting a possible inverse link between osteopontin and the presence of these conditions.

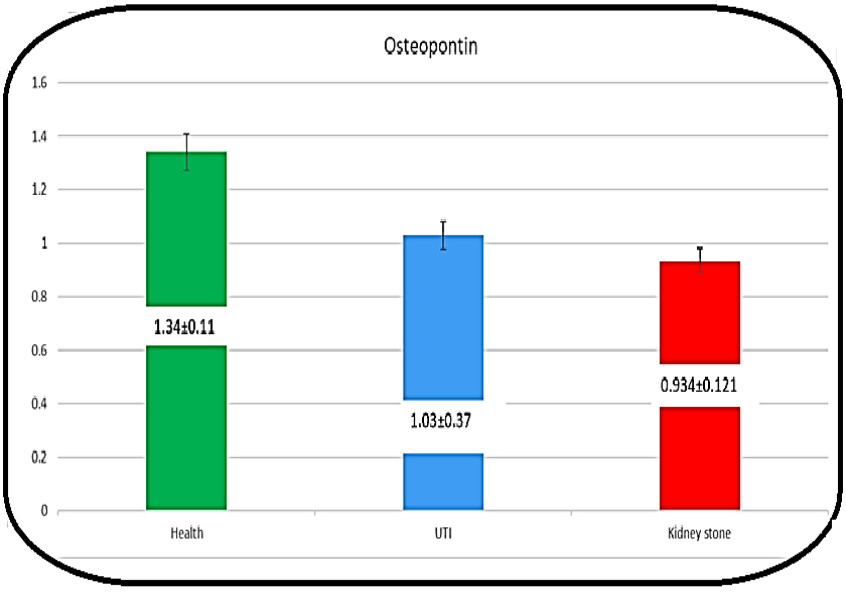

Figure 4. The Concentration of osteopontin among participant.

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According to the cadmium which is one of heavy metals, was used as a biomarker, its concentration was measured only between patients with urolithiasis and healthy individuals, and the results were compared as shown in the (Figure 5) The highest serum cadmium level was found in urolithiasis patients (2.04 ± 0.215 µg/L), while the healthy control had the lowest (0.88 ± 0.121 µg/L) $p<0.001$, indicating a possible link between higher cadmium exposure and urolithiasis development. Cadmium was not assessed in the UTI group because the primary focus was its role as an environmental nephrotoxin in stone formation. Measuring Cd in UTI patients without stones was outside the study scope and not feasible within the available resources.

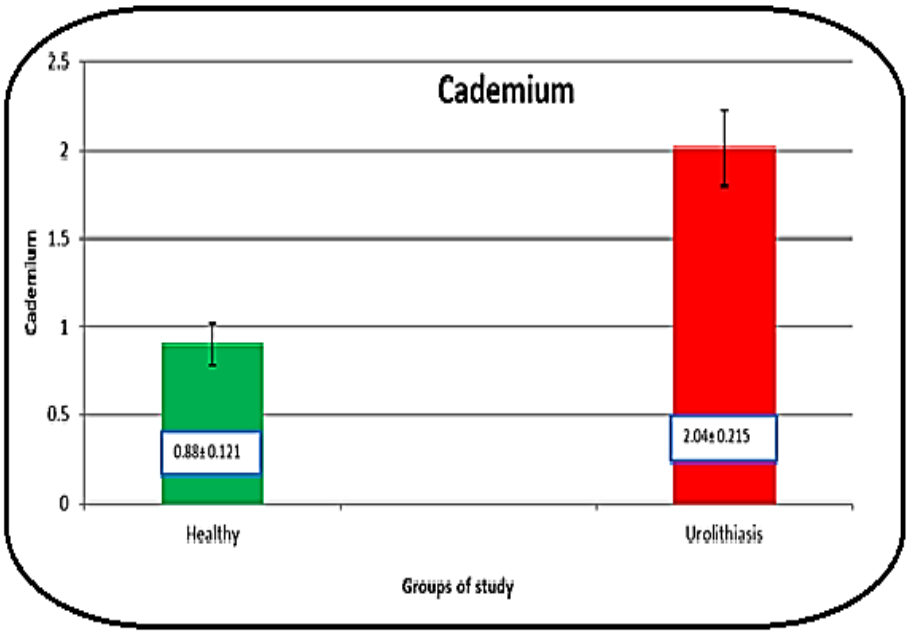

Figure 5. The concentration of cadmium among participant.

Correlation coefficient between immunological markers among patient with Urolithiasis

In Urolithiasis and UTI patients, CA 19-9 showed moderate positive correlations with TLR4 and cadmium, while osteopontin was negatively correlated with both. TLR4 had a strong positive correlation with cadmium, and osteopontin showed a moderate negative correlation with cadmium, all that shown in (Table1). suggesting interconnected marker changes in these conditions.

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Table 1. The correlation coefficient of immunological parameters among urolithiasis and urinary tract infection .

Correlation coefficient	CA 19-9	Toll like receptor 4	Osteopontin	Cadmium
CA 19-9	1			
Toll like receptor 4	0.67361	1		
Osteopontin	-0.56126	-0.79694	1	
Cadmium	0.518894	0.860457	-0.65476	1

Discussion

In this study, no significant differences were observed in age distribution among urolithiasis patients, UTI patients, and healthy controls, indicating comparable demographic backgrounds. This finding is supported by a study published in *BMC Geriatrics* (2022), which reported that age did not significantly differ between patient groups with urolithiasis, suggesting that age is unlikely to confound observed variations in biochemical and immunological markers. However, unmeasured factors such as diet, hydration, smoking, and comorbidities may still influence results and should be considered in future investigations. Serum CA 19-9 levels were significantly elevated in patients with urolithiasis compared to both urinary tract infection (UTI) patients and healthy controls. This finding aligns with previous studies indicating that CA 19-9, although primarily recognized as a tumor-associated glycoprotein, can also rise in non-malignant conditions associated with chronic inflammation or epithelial injury. Specifically, a study conducted in Poland (2019) by Lasota [17], Similarly, a study in **Belgrade, Serbia** by Filipovic [18], described elevated CA 19-9 levels in a patient with benign hydronephrosis caused by obstructive urolithiasis. Serum osteopontin (OPN) levels were significantly reduced in patients with urolithiasis compared to both urinary tract infection (UTI) patients and healthy controls. This decrease aligns with its known role in inhibiting crystal growth and aggregation, suggesting a loss of protective function in stone formation. Similar findings have been reported in previous studies, including research conducted in China (2022) by Jia [19], which highlighted reduced OPN levels in patients with kidney stones, also agree with a study from Japan by Kohri [20], which described the molecular mechanisms by which OPN prevents stone formation. These

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results support the potential use of OPN as a biomarker for urolithiasis risk and may reflect underlying renal stress or injury associated with stone development. TLR4 levels were elevated in both UTI and urolithiasis patients, with the highest in the UTI group, consistent with its role in innate immunity against Gram-negative bacteria. The moderate increase in urolithiasis may reflect sterile inflammation from crystal injury. Differences were statistically significant.These findings are in agreement with previous studies conducted in Japan by Akira [21], highlighted the central role of TLR4 in pathogen recognition and innate immunity, while study in Japan by Kawai [22], confirmed TLR4 upregulation in response to bacterial components, other study by Wang [23], (2016) in China reported increased TLR4 expression associated with kidney stone-induced inflammation. Overall, the current results are compatible with these studies, supporting the idea that TLR4 can be elevated both in infectious and non-infectious (sterile inflammation) conditions of the urinary tract. Cadmium exposure was associated with higher CA 19-9 and TLR4 levels and lower osteopontin levels. These finding align with study by Guo and Järup [24,25], reported that cadmium can promote oxidative stress, disrupt calcium homeostasis, and impair renal tubular function, thereby increasing susceptibility to stone formation. The correlation analysis among the studied immunological and biochemical markers in patients with urolithiasis and urinary tract infection revealed several notable associations. A moderate positive correlation was observed between CA 19-9 and Toll-like receptor 4, suggesting that elevations in one marker tend to be accompanied by increases in the other, possibly reflecting their concurrent involvement in inflammatory processes. These finding agree with Harshita & Kailasa, 2023 who reported that CA 19-9 showed a moderate positive correlation with cadmium levels, indicating a potential link between this tumor-associated glycoprotein and environmental or toxic metal exposure in the context of urinary tract pathology[26]. **Limitations**: The study's cross-sectional design limits causal inference. Environmental exposures, dietary habits, hydration status, and smoking were not systematically assessed. Biomarker measurements were limited to serum, without corresponding urinary analysis or mechanistic experiments. Additionally, the relatively small sample size may reduce generalizability.

Conclusion

The findings suggest that urolithiasis and UTIs are associated with changes in inflammatory and immune biomarkers, potentially influenced by heavy metal exposure. Longitudinal and

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mechanistic studies are required to clarify causal relationships and functional roles of CA 19-9, osteopontin, TLR4, and cadmium in urinary tract pathophysiology.

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