

# An Epidemiological Survey on Kidney Stones and Related Risk Factors in the Iranian Community

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**Abstract-** Increasing number of patients with kidney stones is a major worldwide concern that needs more attention for recognizing the disease in order to set up suitable prevention systems. In this study, we aimed to assess the prevalence and related risk factors of kidney stones in our local area (Isfahan, Iran). In 2011, we celebrated World Kidney Day (WKD) with several training programs for informing people about kidney diseases. A questionnaire containing demographic data, past medical history, and familial and self-history of kidney disease was fulfilled by each individual who participated in WKD. Blood pressure and body mass index (BMI) were also measured using standard methods. Statistical analysis with SPSS-20 software was done. 556 participants with a mean age of  $44.69 \pm 15.32$  were included in the study, of which 107 cases (19.2%) with a mean age of  $50.24 \pm 12.33$  had a kidney stone, and 449 cases (80.8%) with a mean age of  $44.69 \pm 15.32$  had no history of kidney stone. There were no significant differences between those with and without kidney stones regarding sex ( $P=0.176$ ), type of daily work ( $P=0.91$ ), diabetes mellitus ( $P=0.64$ ), and place of living (urban versus rural) ( $P=0.92$ ) and BMI ( $P=0.26$ ). However, there were differences between groups regarding age ( $P<0.001$ ), Hypertension (HTN) ( $P=0.001$ ), Cardiovascular disease (CVD) ( $P=0.02$ ), and familial history of kidney stone ( $P<0.001$ ). Out of 107 patients with kidney stones, the mean number of urinary excretion of stones was  $2.56 \pm 2.98$ . We found a greater prevalence of kidney stones in our local area compared with data from other research studies. Despite some previous studies, comparison of people with and without kidney stones did not reveal differences in the prevalence of DM, type of daily work (low activity versus high activity), and obesity but differences in the field of HTN and CVD were seen.

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**Keywords:** Kidney stone; Risk factors; Iranian community

## Introduction

Kidney stone is a common disorder that causes several troubles, including hypertension (HTN) and financial burden on the health system (1). The risk of disease progression was reported to be 12% and 6% in men and women, respectively (2,3), and two billion dollars is spent on the management of the disease in the United States every year (4), which shows a huge and considerable need

for improvement of preventing systems. Reoccurrence of nephrolithiasis is also a considerable issue, and near to half of the patients with kidney stones are predisposed to new stone formation in a period of ten years (5).

Previous studies revealed an association between the following factors and the incidence of kidney stones: obesity, type 2 diabetes mellitus (DM), insulin resistance, metabolic syndrome, cardiovascular disease (CVD), hypercholesterolemia, poor diet, and genetic factors (6-

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17). Some chemistry changes in urine are responsible for stone formation including hypocitraturia, low urine volume, and hypercalciuria (which may occur as idiopathic hypercalciuria or with hypercalcemia consistent with disorders like hyperparathyroidism, vitamin D excess, sarcoidosis, and malignancies) (5,18,19). There are also some environmental risk factors for kidney stones, such as warm weather (14); workers who tolerate more temperature during daily work are more often complicated by kidney stones compared with others (6).

There are several interventions for the management of kidney stones, but the main aim of all of them is the prevention of new stones formation and prevention of previous stones enlargement (20).

As a rule, epidemiologic studies help investigators to find risk factors for kidney stone disease (21). Data from National Health and Nutritional Examination Survey (NHANES) in 2012 revealed that a total percent of 8.8% (95% confidence interval [CI], 8.1-9.5) of people were affected by kidney stones which consisted of 10.6% (95% CI, 9.4-11.9) in men and 7.1% (95% CI, 6.4-7.8) in women (22). A study of the Chinese population regarding the prevalence of kidney stones also found a percentage of 4.8% in men and 3% in women, with a male predominance of disease occurrence in all groups of age (23). Considering the differences between statistical data of each geographical region and the lack of similar data in our country, in this survey, we would like to find the prevalence of kidney stones in our local area. Evaluating risk factors of kidney stone formation and frequency of interventions for the management of patients with kidney stone disease were other goals of this study.

## Materials and Methods

World kidney day (WKD) is annually celebrated worldwide in order to prevention of kidney-related diseases and also to make the general population informed and aware about the prevention and management of kidney-related disorders. In 2011 Isfahan Kidney Diseases Research Center (IKRC) celebrated the WKD at the central public park (Shahid-Rajaei park; Isfahan, Iran), which is located in the way of schools, universities, offices and etc., in the other hand, people in all range of age, academic degree and other individualized characteristics across mentioned park daily.

During the programs of the celebration, we designed this cross-sectional study to assess the prevalence of kidney stones and some other data about kidney stones in

the general population of our city. The study was granted by the Isfahan University of Medical Sciences. All individuals who participated in the celebration were requested to fulfill a questionnaire containing the following questions:

1-Demographic data (sex and age), 2- the place of residence (Isfahan city, rural area near Isfahan city or other cities), 3- the type of daily work (high versus low activity), 4- familial history of kidney stones (first degree or second degree), 5- the presence of any medical conditions including DM, CVD, and HTN, 6- weekly usage of vitamin C and vitamin D, 7- the presence of kidney stone disease in yourself? (if yes, reply to the below questions), 8- number of urinary excretion of stones, 9- number of interventions done for resolving kidney stone disease (extracorporeal shock wave lithotripsy, surgery, medication and etc.), 10- frequency of visiting your physician for kidney stone related problems and complications, 11- what is your physician specialty, 12- frequency of urinary system imaging [kidney ureter and bladder (KUB) x-ray, computed tomography scan (CT scan), sonography and etc.]

We requested participants to sit down during answering the questions and not drink caffeine-saturated materials or do not smoke cigarettes. After that, medical students who studied at university for more than four years measured each person's height, weight, and systolic and diastolic blood pressure using a standard blood pressure cuff instrument (MDF, USA). The body mass index (BMI) was measured by weight (kilograms) over height (meters) squared.

Questionnaires which was unreadable or had incomplete data were excluded from the study, then gathered data were analyzed using SPSS-20 software (SPSS Inc., Chicago, Illinois, USA). T-test and Pearson chi-square test were used for data analysis, and *P* less than 0.05 was considered a statistically significant difference.

## Results

Five hundred fifty-six participants were included in the study. Demographic data are shown in table 1. Out of all patients with a kidney stone, 91 patients (85%) and 16 patients (21%) had low activity and high activity daily work, respectively. There were 389 individuals (86.6%) who had low activity work and 60 individuals (13.4%) who had high activity daily work in the group of individuals without kidney stones. Pearson chi-square test did not reveal differences between groups in the field of daily work (*P*=0.91).

**Table 1. Demographic data and BMI of participants**

| History of kidney stone                |        | Yes         | No          | Total       | P      |
|--|--------|-------------|-------------|-------------|--------|
| Total number of participants (percent) |        | 107 (19.2%) | 449 (80.8%) | 556 (100%)  | -      |
| Gender                                 | Male   | 64 (21.3%)  | 236 (78.7%) | 300 (100%)  | 0.176  |
|  | Female | 43 (16.8%)  | 213 (83.2%) | 256 (100%)  |        |
| Average age                            |        | 50.24±12.33 | 43.37±15.68 | 44.69±15.32 | <0.001 |
| BMI                                    |        | 26.96±4.60  | 26.36±5.05  | 26.48±4.97  | 0.26   |

-BMI: Body mass index

Statistical analysis showed no difference between groups in place of living, daily work, diabetes mellitus, and vitamin consumption. As seen in Table 2, a

significant difference was seen between groups with and without kidney stones regarding the familial history of the disease.

**Table 2. Probable risk factors of kidney stone**

| Variables                        | History of kidney stone |             | Total      | P          |        |
|----------------------------------|-------------------------|-------------|------------|------------|--------|
|                                  | Yes                     | No          |            |            |        |
| Daily work                       | High activity           | 16(21%)     | 60(13.4%)  | 76(13.7%)  | 0.91   |
|                                  | Low activity            | 91(85%)     | 389(86.6%) | 480(86.3%) |        |
|                                  | Isfahan (Urban area)    | 64(59.8%)   | 271(60.3%) | 335(60.3%) |        |
| Place of living                  | Rural area near Isfahan | 37(34.6%)   | 157(35%)   | 194(34.9%) | 0.92   |
|                                  | Other cities            | 6(5.6%)     | 21(4.7%)   | 27(4.8%)   |        |
|                                  | Diabetes Mellitus       | Present     | 23(21.5%)  | 64(14.2%)  |        |
|                                  | Absent                  | 84(78.5%)   | 385(85.8%) | 469(84.4%) |        |
| Cardiovascular diseases          | Present                 | 13(12.1%)   | 26(5.8%)   | 39(7%)     | 0.02   |
|                                  | Absent                  | 94(87.9%)   | 423(94.2%) | 517(93%)   |        |
| Hypertension                     | Present                 | 36(33.6%)   | 86(19.1%)  | 122(21.9%) | 0.001  |
|                                  | Absent                  | 71(66.4%)   | 363(80.9%) | 434(78.1%) |        |
| Vitamin C consumption            | Yes                     | 2 (1.9%)    | 9(2.1%)    | 11(1.9%)   | 0.21   |
|                                  | No                      | 105 (98.1%) | 440(97.9%) | 545(98.1%) |        |
| Vitamin D consumption            | Yes                     | 2(1.9%)     | 11(2.5%)   | 13(2.3%)   | 0.13   |
|                                  | No                      | 105(98.1%)  | 438(97.5%) | 543(97.7%) |        |
| Familial history of kidney stone | No                      | 57(53.3%)   | 343(76.4%) | 400(71.9%) | <0.001 |
|                                  | First degree            | 46(43%)     | 93(20.7%)  | 139(25%)   |        |
|                                  | Second degree           | 4(3.7%)     | 13(2.9%)   | 17(3.1%)   |        |
| Total                            |                         | 107(19.2%)  | 449(80.8%) | 556(100%)  | -      |

There were just 23 participants who knew that he/she had HTN, of which just 5 of them were controlled. Measurement of blood pressure by medical students revealed significant differences with subjective data of patients, meaning that there were 122 cases of HTN (including stage 1 and stage 2 of HTN, but not pre-HTN cases) and 99 out of 122 patients (81.14%) did not know about their elevated levels of blood pressure. Statistical analysis revealed differences between groups with and without kidney stones regarding the prevalence of HTN (33.64% versus 19.15%;  $P=001$ ).

Out of 107 patients with kidney stones, the mean number of urinary excretion of stones was  $2.56\pm 2.98$ . The number of patients who underwent extracorporeal shock wave lithotripsy and surgery was 29 (27.1%) and 22 (20.5%), respectively. About one-third of cases with a

kidney stone (33 out of 107; 30.8%) did not meet a physician for their kidney-related disorders at any time, and 14 cases (13.1%) did not have a regular programmed visiting period. Sixteen (15%) and seventeen (15.9%) of them visited physicians monthly and bimonthly, respectively. We categorized physicians' specialties each individual as the following: nephrologist (56 cases; 52.3%), urologist (18 cases; 16.8%), not known (17 cases; 15.9%), and others including internist, general practitioner, etc. (16 cases; 15%).

A great number of patients (55 cases; 51.4%) did not have medical imaging of the urinary system at any time; however, in the others who had imaging, most of them were referred for urinary system imaging (Sonography, computed tomography scan, kidney ureter bladder x-ray and etc.) every six months (19 cases; 17.8% of all kidney

stone cases). Fifty-one patients with a kidney stone (47.66%) had routine annual imaging related to their disease. Eighty one patient with a kidney stone (85%) did not use any medication in order to manage the management of kidney stone, and the others used "not known" drug (4.7%) and potassium citrate (10.2%) for the same reason.

## Discussion

In this study, we evaluated the prevalence of kidney stones, related risk factors, and frequency of interventions for the management of the disorder. A study by Bihl *et al.*, showed the prevalence of kidney stones as 15% in men and 6% in women of Caucasian race (24). In contrast, our data revealed a total percent of 19.2% of people who were involved by kidney stones, and a subsequent division regarding sex showed that 21.3% of men and 16.8% of women were kidney stone formers. The etiology of this interesting and different pattern of disease prevalence between Iranian and NHANES data (22) can be an issue for potential further studies. Our data showed that the mean age of our patients with kidney stones were  $50.24 \pm 12.33$  showing that the disease liked to involve middle-aged people; similar to our findings, a study in our local area which investigated the metabolic disorders in 437 cases of the renal stone showed a mean age of  $46 \pm 13.8$  years of participants (25). A review article concluded that since there is a relationship between DM and uric acid lithiasis, not enough pieces of evidence are present, suggesting that a higher prevalence of calcium lithiasis is present in patients with DM (26). In contrast, DM and obesity were reported to be more prevalent among patients with kidney stones compared with people without kidney stones in the NHANES study (22). Our data did not show more cases of DM in groups of kidney stone formers, and the BMI had no difference between groups.

Among risk factors of coronary heart disease, HTN and hyperlipidemia are the most commonly related risk factors of urinary stones (27-29); There was an increased number of CVD and HTN in our kidney stone patients compared with people without kidney stones.

Considering dietary regimen, a high intake of vitamin C is a possible predisposing factor for kidney stones; however, no proven association was documented in mentioned regard (30). The maximum allowed intake dose of vitamin C is 2000 mg daily (30). Vitamin C is absorbed in the gastrointestinal tract and would convert to oxalate (a principal material of kidney stone's formulation) (31) that deliver into the bloodstream and

lead to oxaluria (32-34). Genetic variations may result in different metabolic pathways of vitamin C (35) that may explain the possible association between Vitamin C intake and kidney stone formation. A study by Grases *et al.*, in Spain, showed that men who intake more than one gram of Vitamin C per day were at a higher risk of 40% for urinary stone formation compared with those who intake vitamin C in lesser amounts (36). Because we did not have enough cases who consumed vitamin C and D in both groups, our data did not help us to conclude a strong relationship between vitamin intake and the formation of kidney stones.

A previous study in our country-Iran-showed history found that 28.6% of patients with kidney stones had a familial history of the same disorder. Their data revealed that the role of familial history in the self progression of the disease is more prominent in men compared with women. They also found that there was no relationship between age of disease onset and the presence of the familial history of the disease in men who were involved by renal calculi (37). Our data revealed that 46.7% of those patients with kidney stones had a positive familial history (first degree and second degree together) of kidney stones. However, there were just 23.6% of those patients without kidney stones had a positive familial history of kidney stones. A significant statistical difference in the above subjects suggested that according to previous studies, familial history of kidney stones is considered a risk factor for disease progression.

Recently, Goldfarb *et al.* hypothesized a relationship between the increasing prevalence of kidney stones and worldwide urbanization and exposure to urban heat islands (38). In a similar way, most of our cases who had renal stone was living in urban areas, but our data did not reveal the difference between people with and without renal stones regarding the living place.

We would like to categorize the type of medications used for the treatment of renal stones, but a huge limitation was seen due to patients' lack of knowledge about their medications. As we asked patients for their medications, the most heard answer was, "There was a drug which I previously used, but I do not remember its name," meaning that most of the cases did not have enough data about the necessity of treatments. During past decades the general trend of physicians for treatment of kidney stones has been using minimal invasive manners including percutaneous nephrolithotomy, shock wave lithotripsy (the most common way of treatment), retrograde internal surgery, and ureteroscopy (27). Our data also revealed that more people experienced extracorporeal shock wave lithotripsy rather than

invasive interventions like surgery.

We did not have enough data about the molecular type of kidney stones in our participants, which can be considered a limitation of our study. Other limitations of our study may be the lack of enough knowledge of patients about their medications, imaging modalities, and plan of treatment for their disease. Regarding our higher prevalence of kidney stones, it is beneficial to say that we examined people who participated in WKD, and the mentioned group may cause a bias in study results. It would be probable that people with kidney diseases were more eager to participate in WKD, and this issue could be the leading cause of the higher prevalence of kidney stones in our population. Another disturbing variable of our study was the hot and dry weather of our area, which may affect the prevalence of kidney stones in our locals.

In conclusion, we found a greater prevalence of kidney stones in our local area compared with data from other research studies. Despite some previous studies, comparison of people with and without kidney stones did not reveal differences in the prevalence of DM, type of daily work (low activity versus high activity), and obesity but differences in the field of HTN and CVD were seen.

## References

- Cupisti A, Morelli E, Meola M, Cozza V, Parrucci M, Barsotti G. Hypertension in kidney stone patients. *Nephron* 1996;73:569-72.
- Radimer K, Bindewald B, Hughes J, Ervin B, Swanson C, Picciano MF. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999-2000. *Am J Epidemiol* 2004;160:339-49.
- Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. *Kidney Int* 2003;63:1817-23.
- Akoudad S, Szklo M, McAdams MA, Fulop T, Anderson CA, Coresh J, et al. Correlates of kidney stone disease differ by race in a multi-ethnic middle-aged population: the ARIC study. *Prev Med* 2010;51:416-20.
- Han H, Segal AM, Seifter JL, Dwyer JT. Nutritional Management of Kidney Stones (Nephrolithiasis). *Clin Nutr Res* 2015;4:137-52.
- Atan L, Andreoni C, Ortiz V, Silva EK, Pitta R, Atan F, et al. High kidney stone risk in men working in steel industry at hot temperatures. *Urology* 2005;65:858-61.
- Curhan GC, Rimm EB, Willett WC, Stampfer MJ. Regional variation in nephrolithiasis incidence and prevalence among United States men. *J Urol* 1994;151:838-41.
- Curhan GC, Willett WC, Rimm EB, Speizer FE, Stampfer MJ. Body size and risk of kidney stones. *J Am Soc Nephrol* 1998;9:1645-52.
- Curhan GC, Willett WC, Rimm EB, Stampfer MJ. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* 1993;328:833-8.
- Daudon M, Traxer O, Conort P, Lacour B, Jungers P. Type 2 diabetes increases the risk for uric acid stones. *J Am Soc Nephrol* 2006;17:2026-33.
- Edvardsson VO, Palsson R, Indridason OS, Thorvaldsson S, Stefansson K. Familiality of kidney stone disease in Iceland. *Scand J Urol Nephrol* 2009;43:420-4.
- Sakhaee K. Nephrolithiasis as a systemic disorder. *Curr Opin Nephrol Hypertens* 2008;17:304-9.
- Siener R. Impact of dietary habits on stone incidence. *Urol Res* 2006;34:131-3.
- Soucie JM, Coates RJ, McClellan W, Austin H, Thun M. Relation between geographic variability in kidney stones prevalence and risk factors for stones. *Am J Epidemiol* 1996;143:487-95.
- Taylor EN, Stampfer MJ, Curhan GC. Diabetes mellitus and the risk of nephrolithiasis. *Kidney Int* 2005;68:1230-5.
- Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. *JAMA* 2005;293:455-62.
- Sakhaee K, Maalouf NM. Metabolic syndrome and uric acid nephrolithiasis. *Semin Nephrol* 2008;28:174-80.
- Kok DJ, Iestra JA, Doorenbos CJ, Papapoulos SE. The effects of dietary excesses in animal protein and in sodium on the composition and the crystallization kinetics of calcium oxalate monohydrate in urines of healthy men. *J Clin Endocrinol Metab* 1990;71:861-7.
- Lemann J, Jr., Worcester EM, Gray RW. Hypercalciuria and stones. *Am J Kidney Dis* 1991;17:386-91.
- Asplin JR. Evaluation of the kidney stone patient. *Semin Nephrol* 2008;28:99-110.
- Matlaga BR, Schaeffer AJ, Novak TE, Trock BJ. Epidemiologic insights into pediatric kidney stone disease. *Urol Res* 2010;38:453-7.
- Scales CD Jr, Smith AC, Hanley JM, Saigal CS, Urologic Diseases in America P. Prevalence of kidney stones in the United States. *Eur Urol* 2012;62:160-5.
- Zeng Q, He Y. Age-specific prevalence of kidney stones in Chinese urban inhabitants. *Urolithiasis* 2013;41:91-3.
- Bihl G, Meyers A. Recurrent renal stone disease-advances in pathogenesis and clinical management. *Lancet* 2001;358:651-6.
- Emami-Naini A, Eshraghi A, Shahidi S, Mortazavi M, Seyrafi S, Roomizadeh P, et al. Metabolic evaluation in patients with nephrolithiasis: A report from Isfahan, Iran.

- Adv Biomed Res 2012;1:65.
26. Arrabal-Polo MA, Arrabal-Martin M, Garrido-Gomez J. Calcium renal lithiasis: metabolic diagnosis and medical treatment. *Sao Paulo Med J* 2013;131:46-53.
  27. Mehmet NM, Ender O. Effect of urinary stone disease and its treatment on renal function. *World J Nephrol* 2015;4:271-6.
  28. Ando R, Nagaya T, Suzuki S, Takahashi H, Kawai M, Okada A, et al. Kidney stone formation is positively associated with conventional risk factors for coronary heart disease in Japanese men. *J Urol* 2013;189:1340-6.
  29. Jungers P, Joly D, Barbey F, Choukroun G, Daudon M. ESRD caused by nephrolithiasis: prevalence, mechanisms, and prevention. *Am J Kidney Dis* 2004;44:799-805.
  30. Massey LK, Liebman M, Kynast-Gales SA. Ascorbate increases human oxaluria and kidney stone risk. *J Nutr* 2005;135:1673-7.
  31. Urivetzky M, Kessarid D, Smith AD. Ascorbic acid overdosing: a risk factor for calcium oxalate nephrolithiasis. *J Urol* 1992;147:1215-8.
  32. Baxmann AC, De OGM, Heilberg IP. Effect of vitamin C supplements on urinary oxalate and pH in calcium stone-forming patients. *Kidney Int* 2003;63:1066-71.
  33. Chalmers AH, Cowley DM, Brown JM. A possible etiological role for ascorbate in calculi formation. *Clin Chem* 1986;32:333-6.
  34. Traxer O, Huet B, Poindexter J, Pak CY, Pearle MS. Effect of ascorbic acid consumption on urinary stone risk factors. *J Urol* 2003;170:397-401.
  35. Briggs M. Letter: Vitamin-C-induced hyperoxaluria. *Lancet* 1976;1:154.
  36. Grases F, March JG, Prieto RM, Simonet BM, Costa-Bauza A, Garcia-Raja A, et al. Urinary phytate in calcium oxalate stone formers and healthy people--dietary effects on phytate excretion. *Scand J Urol Nephrol* 2000;34:162-4.
  37. Ahmadi Asr Badr Y, Hazhir S, Hasanzadeh K. Family history and age at the onset of upper urinary tract calculi. *Urol J* 2007;4:142-5.
  38. Goldfarb DS, Hirsch J. Hypothesis: Urbanization and exposure to urban heat islands contribute to increasing prevalence of kidney stones. *Med Hypotheses* 2015;85:953-7.