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# Influence of Water and Food Quality on Renal Stones Disease

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**ABSTRACT**

Kidney stones are a common health issue affecting many individuals worldwide, causing severe pain and potentially impacting the quality of life. Among the primary factors contributing to the formation of kidney stones drinking water and food play a significant role. While many people may associate kidney stone formation primarily with genetic factors or chronic diseases, the quality of water consumed and the foods ingested are crucial factors in triggering this condition. The concentration of salts and minerals in water along with the types of foods consumed, are closely linked to an increased risk of kidney stone formation. Therefore, understanding these factors is essential for deeper insight and the development of effective preventive measures. Investigate the impact of drinking water and food on the formation of kidney stones. Specifically, it aims to explore how the composition of water including its mineral and salt content, along with dietary habits influence the risk of kidney stone development. In addition to understanding these factors the study will provide insights into effective prevention strategies and raise awareness about the importance of water quality and nutrition in maintaining kidney health.

**Key Words:** drinking water, renal stones, Oxalate food, pH, alkalinity, hardness, food.

## INTRODUCTION

Kidney stones are a common medical condition that can cause significant pain and discomfort affecting millions of individuals worldwide. The formation of kidney stones occurs when certain substances in the urine, such as calcium, oxalate and uric acid become concentrated and crystallize. While genetic factors and pre-existing medical conditions like hyperparathyroidism, obesity, and urinary tract infections are known to contribute to the formation of kidney stones lifestyle factors especially diet and hydration play a critical role in their development. One of the most important environmental factors influencing kidney stone formation is the quality of drinking water. The mineral composition of drinking water including the levels of calcium, magnesium and other dissolved salts can significantly impact the likelihood of developing kidney stones. Hard water which contains higher levels of calcium and magnesium have been shown to increase the risk of stone formation particularly in regions where people do not consume adequate water. On the other hand, soft water which typically has lower mineral content may not present the same risks. Research has shown that adequate hydration can help dilute the concentration of stone forming substances in urine reducing the likelihood of crystal formation. A study concluded that increasing fluid intake is one of the most effective preventive measures against kidney stones [1]. Dietary factors are also crucial in determining the risk of kidney stone formation. A diet high in animal proteins, sodium, and oxalate rich foods can increase the likelihood of stone formation. Animal proteins for instance increase calcium excretion in urine while high sodium intake leads to greater calcium excretion both of which promote the formation of calcium-based stones. Additionally, certain foods such as spinach, watercress, and okra which are rich in oxalates can increase urinary oxalate levels raising the risk of calcium oxalate stone formation. Conversely, a diet high in fruits and vegetables which are rich in potassium and citrate can help prevent kidney stones by promoting a more alkaline urine environment, which inhibits stone formation. Recent studies such as those conducted by [2], have shown that dietary patterns high in fruits and vegetables and low in animal protein and sodium can significantly reduce the risk of stone formation [3]. In addition to these dietary and water related factors lifestyle behaviors such as physical activity also play a role in kidney stone prevention. Sedentary individuals tend to have a higher risk of developing kidney stones as physical inactivity can reduce urine output and contribute to the accumulation of minerals in the kidneys. On the other hand, regular physical activity promotes healthy hydration and increases urine flow both of which help to prevent stone formation. Preventing kidney stones involves not only understanding the role of water and diet but also adopting a holistic approach that includes proper hydration a balanced diet and an active lifestyle. Making informed choices about the food we consume and ensuring that we maintain adequate hydration we can significantly reduce the risk of developing kidney stones. Continued research into the links between diet, water quality, and kidney stone formation will provide further insight into effective preventive strategies offering hope for reducing the global prevalence of this painful condition.

**OBJECTIVES OF The STUDY**

1-Assess the relationship between drinking water quality and food with kidney stone formation: Measure the concentrations of minerals such as calcium, magnesium, and oxalates in drinking water samples from various regions. Analyze the correlation between these mineral levels and the

incidence rates of kidney stones in the corresponding populations.

2- Evaluate the impact of dietary habits on kidney stone formation: Collect dietary intake Data focusing on food high in oxalates, and sodium among a selected group of individuals. Compare these dietary patterns with the prevalence of kidney stones within the same group3- Investigate the Impact of smoking on kidney stone formation: Examine the association between smoking and the increased risk of developing kidney stones. Analyze how smoking affects kidney function and contributes to the deterioration of renal health. Compare the incidence rates of kidney stones between smokers and non-smokers. Research indicates that smoking can adversely effect on kidney health.

4- Develop preventive recommendations based on study findings: Formulate dietary guidelines aimed at reducing the consumption of foods that may contribute to kidney stone formation. Provide evidence-based hydration advice to assist individuals in minimizing their risk of developing kidney stones.

5- Assess heavy metal concentrations in renal stones samples: Quantify levels of heavy metals such as lead, cadmium, and iron in kidney stones samples from patients with nephrolithiasis. Compare these concentrations with those found in individuals without kidney stones to identify significant differences.

6- Provide evidence-based hydration advice to assist individuals in minimizing their risk of developing kidney stones.

**MATERIAL AND METHODS**

**1-Sample Collection**

**1.1-Food samples**

Ten vegetable samples were selected from the local market of Zagazig city for determination of oxalate content. The selected samples were Spinach, watercress, okra, parsley, cabbage, zucchini, tomato, Cucumber, potatoes, and carrot. All the selected samples of vegetables were packed in a clean Polyethylene bag and transferred to laboratory for processing. The samples were divided into two parts, 100g of clean sample was dried directly in an oven at a temperature of (45°C) and another 100g of sample was boiled for 15 minutes in fresh water then the samples were kept in oven for drying at temperature of (45°C) for 24 hours. After drying all the samples were pulverized to a uniform particle size and packed in an airtight bottle for further analysis. Samples were analyzed using titration method against $KMnO_4$ in the determination of oxalate where1g of each selected vegetable samples were weighted and mixed with 20 ml 0.1M HCl in a 50ml beaker to extract total oxalate and another 1g of each selected samples were weighed and mixed with 20ml of distilled water to extract soluble oxalate. All beakers with samples and extracting solvents were kept in a water bath at 100°C for 30 minutes later filtrated using Watt man No, 1 filter paper. The 0.5ml of 5% Calcium chloride was added to the filtrate to precipitate out Calcium oxalate the precipitate was separated by centrifugation at 3500 rpm for 15 minutes and supernatant was discarded. The Calcium oxalate precipitate was washed with 2ml of 0.35M Ammonium hydroxide and then dissolved in 0.5M of Sulphuric acid. The dissolved solution was titrated with 0.1M of Potassium Permanganate at 60°C till faint pink color was persisted for at least 15 seconds. The oxalate content was calculated by using stoichiometric formula. The soluble oxalate was subtracted from total oxalate to obtain insoluble oxalate. Both soluble and insoluble oxalates were expressed on dry weight basis (DW) [4].

1.2-**Water samples**

The water samples were collected from the sources that kidney stone patient's use for drinking in order to analyze the water's properties and its potential impact on their health. The drinking water samples were analyzed for pH, alkalinity, hardness, total dissolved solids (TDS), electrical conductivity (EC), and temperature. The alkalinity and hardness were determined by the titration method using hydrochloric acid and ethylene diamine tetra acetic acid while PH, TDS, EC, and temperature were measured by using instruments.

The drinking water samples were collected from various sources commonly used by kidney stone Patients. The sampling was conducted following standard procedures to ensure accurate and reliable results. Samples were gathered in sterilized bottles to avoid contamination and proper labels were placed with details such as the source, date, and time of collection. The samples were transported to the laboratory under appropriate conditions to maintain their integrity until analysis and stored in refrigerator until analyzed in our laboratory within 24 hours. [5, 6]

**1.3- Analysis of water samples by using AAS**

**Measurement of Ca concentration in drinking water**

Atomic Absorption Spectroscopy (AAS) is commonly used for measuring the concentration of calcium (Ca) in drinking water due to its high sensitivity and specificity for metal ions. The most common wavelength for Calcium in (AAS) is 422.7 nm but these can very slightly depend on the instrument and lamp used. The atomic absorption spectrometer used in this study is a SavantAA model, manufactured by GBC Scientific Equipment Ltd an analytical instrument company based in Victoria, Australia. The instrument is a double-beam atomic absorption spectrometer (AAS) designed for accurate trace metal analysis. The serial number of the instrument is A7322GBC and its code number is 20. The device was manufactured in Australia.

**Sample preparation**

Method for separation of metals in drinking water using cationic-exchange column with cation resin in the presence of nitric acid. The cationic-exchange column resin method is an effective technique for separating metals from drinking water. It relies on the interaction between cations (such as heavy metals or other elements in the water) and cation resin. Nitric acid cans elution of metals ions from resin.

**1. Preparation of the cationic -Exchange Column**: Column Setup: The cation-exchange column is typically a long glass tube filled with cation resin material.

**2. Preparation of the Drinking Water Sample:** A sample of drinking water containing the metals to be separated is collected. These metals may include calcium, magnesium, iron, and other heavy metals.

**3. Separation Process: Loading the Sample:** After preparing the sample, it is slowly added to the cation-exchange column packed with cation resin. One liter of water sample is filtered to remove any particulates then using separation column and cation resin. Filled the separation column by cation resin at 1/3 higher of column, then flow the water sample on resin and optimum flow rate at 1.5 ml /min. After finishing the flowing add 100ml of 2N nitric acid (HNO3) on resin to make recovery of resin. Take the filtrate to determine the heavy metals on Atomic Absorption Spectroscopy (AAS). As the sample passes through the column the positively charged metal ions interact with the cation resin inside the column. Metals that bind more strongly to the cation resin will remain at the top of the column while those that interact less strongly will flow through the column more quickly. Washing the Column after loading the sample the column is washed with nitric acid to release the ions bound to the cation resin. This step ensures the separation of metals and their collection [7,8].

**4. Collection of Separated Metals:** The metals that have been separated are collected in bottles as they are eluted from the column. Analytical techniques such as Atomic Absorption Spectroscopy (AAS) can then be used to analyze the metal content in the collected samples providing the concentration of metals in the drinking water.

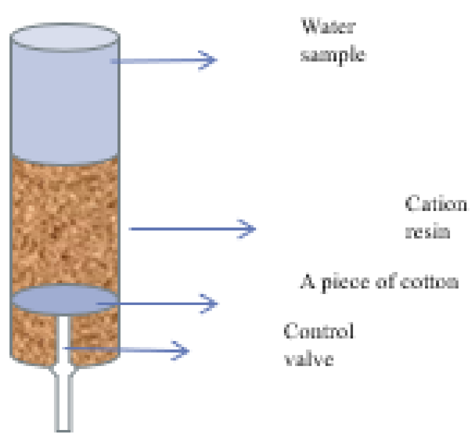


**Fig (1) Cation Exchange Separation Column**

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**5. Regeneration of Resin:** After separating the metals from the column the remaining cations can be recovered by using a solution with a higher concentration of nitric acid (100ml of 2N HNO3). This step allows the cationic exchange to be reused in future separations [7, 8].

**1.4-Stones sample**

**A-Selection of patients**

Patients admitted in the department of urology and the diagnosis of them which revealed of stones diseases. And the Patients numbers (n=50) in the department of Urology of university hospital in Zagazig city that confirmed by examining them from the department Urology but information about age, gender, residence, and source of drinking water were collected from the study participants through a questionnaire. Also checked the participants, clinical profiles and studied parameters as calcium oxalate, phosphate, Carbonate and urate salts from urine samples and stones.

**B-Sample preparation;**

**Grinding:** stones are typically grinding into a fine powder for analysis this increases the surface area for inter action with IR light.

**KBr method**: Small amount of powder stones can be mixed with potassium bromide (KBr) and then pressed into a disk. Potassium bromide (KBr) is commonly used in infrared (IR) spectroscopy for the analysis of urinary stones due to its ability to enhance the clarity of IR spectral data. It acts as a dispersing agent in the preparation of KBr pellets which are utilized to analyze the infrared absorption spectra of various materials including kidney stones. The KBr matrix allows the sample to be effectively compressed into a transparent pellet without interfering with the infrared absorption of the sample itself. When a stone sample is analyzed using IR spectroscopy the infrared radiation passes through the sample and the specific absorption bands correspond to different molecular vibrations. Potassium bromide is chosen because of its wide transparency window in the IR region making it ideal for preparing samples without introducing any significant absorption that could mask or distort the stone's spectral profile.

**Kidney Stones: Collection, Classification, Size, Color, and Types for kidney stones**

Kidney stones are hard deposits formed from minerals and salts in the kidneys. They can vary in size from tiny crystals to large stones that may cause significant pain. Kidney stones are a common medical condition that often requires both diagnostic and therapeutic interventions [9].

**The process of diagnosing kidney stones involves collecting stone samples classifying them based on their chemical composition, size, color, and type:**

**1. Collection of Kidney Stone Samples:**

**Kidney stones can be collected using several methods depending on the size and location of the stone:**

**Surgical extraction**: In cases where the stones are too large or cause an obstruction surgical intervention may be necessary to remove them. **Imaging techniques**: X-rays, ultrasound, and MRI( Magnetic Resonance Imaging) can be used to locate and identify stones within the kidneys.

**2. Classification of Kidney Stones:**

Kidney stones are classified based on their chemical composition:

**Calcium Stones**: **Calcium oxalate stones**

These are the most common type of kidney stones and form when calcium binds with oxalate in the urine.

**Calcium phosphate stones**

These stones form in more alkaline (basic) urine and are often associated with chronic urinary tract infections or certain metabolic conditions.

**Uric Acid Stones**

These stones form when there are high levels of uric acid in the urine typically seen in individuals with gout or high levels of uric acid in the blood.

**Struvite Stones**

These are less common and usually form in the presence of urinary tract infections (UTIs) caused by bacteria that produce ammonia which raises the urine pH leading to stone formation.

**Cystine Stones**

Rare stones caused by a genetic disorder that leads to an excess of the amino acid cystine in the urine.

**3. Size and Color of Kidney Stones:**

**Size:** Kidney stones can vary greatly in size from as small as 1 mm (which may pass unnoticed) to larger stones over 2 cm in diameter which may require medical intervention like shockwave therapy or surgery.

**Color:**

**White or gray**: Often indicates calcium phosphate or struvite stones in fig (2,3).

Fig (2) **white stone**

**Fig (3) gray stone**

**Dark brown or yellow**: **Typically associated with calcium oxalate stones in fig (4,5).**

**Fig (4) dark brown stone**

**Fig (5) yellow stone**

**Black: Rare and may indicate cystine stones in fig (6,7)**.

**Fig (6) black stone**

**Fig (7) black stone**

**Light yellow**: **Associated with uric acid stones in fig (8,9).**

**Fig (8) light yellow stone**

**Fig (9) yellow stone**

**4. Factors Contributing to Kidney Stone Formation:**

**Several factors contribute to kidney stone formation including:**

**Genetics:** A family history of kidney stones increases the likelihood of developing them.

**Dehydration:** Insufficient water intake can concentrate urine which increases the chance of stone formation.

**Dietary factors**: High intake of foods rich in oxalates, calcium, or animal proteins can contribute to stone formation.

**Medical conditions**: Conditions like gout, hypertension, diabetes, and certain digestive diseases can increase the risk of developing kidney stones.

**5**. **Diagnosis of Kidney Stones**: Diagnosis involves several techniques:

**Clinical examination:** Patients often present with symptoms such as severe flank pain, blood in the urine, and frequent urination.

**Imaging studies**: X-rays, ultrasounds, or MRI( Magnetic Resonance Imaging) are used to detect stones and determine their size and location.

**Urine analysis**: Helps to detect the presence of crystals or red blood cells which indicate kidney stone formation.

**6. Prevention of Kidney Stones**:

**Increase fluid intake:** Drinking adequate amounts of water helps dilute urine and prevent stone formation.

**Reduce sodium and oxalate-rich foods**: Limiting foods like spinach, nuts, and Okra may help reduce the risk of calcium oxalate stones.

**Consume foods rich in citrate**: Citrusy fruits like oranges and lemons can help reduce the risk of stone formation as they inhibit crystal formation.

**Limit animal protein intake:** High intake of animal protein can increase uric acid levels and contribute to uric acid stones. [9].

**C-Analysis of stones samples:**

Stones samples were collected and analyzed by IR instrument spectroscopy, Model Alpha 11 (PN: 1010948\20) Broker Opie GmbH Ruud off -plank.IR instrument was analyzed stones samples by using infrared (IR) spectroscopy with potassium bromide. Infrared (IR) spectroscopy is a widely used and effective method for identifying the chemical composition of kidney stones collected from patients. This technique relies on measuring the infrared absorption spectra of a sample which reveals the molecular structure of the components. When potassium bromide (KBr) is added to the sample it helps improve the clarity of the spectra by reducing moisture absorption which can interfere with the analysis.

**Analysis of kidney stone Method Using IR Spectroscopy:**

**1. Sample Preparation:** Kidney stones collected from patients are first ground into a fine powder. The powdered stone is then mixed with potassium bromide (KBr) in a specific ratio. KBr helps enhance the clarity of the spectra and ensures a homogeneous sample for analysis.

**2. Analysis of kidney stone Using the IR Spectrometer:** The sample mixed with KBr is prepared in compressed discs under high pressure. The sample is then scanned using an IR spectrometer over the mid-infrared range (4000 to 400 $cm^{-1}$), where the absorption bands corresponding to various chemical bonds in the kidney stone are recorded **[10].**

Data about patients (age- gender- residence- food habit- diet) and source of drinking water through a questionnaire.

**2-RESULT AND DISCUSSION**

**2.1-Food samples results:** The total oxalate, soluble oxalate and insoluble oxalate content of the selected ten samples were expressed in mg/100g of dry weight. The oxalate content of unboiled and boiled samples with percentage of reduction is given in table 2.

**Table1: Names of plants food samples which are investigated in the present study**

| No. | Plant Name | Part of Plant |
|---|---|---|
| 1- | Watercress | Stem, leaves |
| 2- | Okra | Fruit vegetables |
| 3- | Parsley | Stem, leaves |
| 4- | Cabbage | Leaves |
| 5- | Zucchini | Fruit vegetables |
| 6- | Tomato | Fruit vegetables |
| 7- | Cucumber | Fruit vegetables |
| 8- | Potatoes | Root vegetables |
| 9- | Carrot | Root vegetables |
| 10- | Spinach | Stem, leaves |

**Table 2: Oxalate content in selected raw vegetable samples on dry weight basis.**

| | | Un boiled samples mg/100g | | | boiled samples mg/100g | | |
|---|---|---|---|---|---|---|---|
| No. | Plant Name | Total Oxalate | Soluble Oxalate | insoluble Oxalate | Total Oxalate | Soluble Oxalate | insoluble Oxalate |
| 1 | watercress | 748 | 660 | 88 | 528 | 440 | 88 |
| 2 | Okra | 660 | 528 | 132 | 440 | 396 | 44 |
| 3 | Parsley | 528 | 440 | 88 | 352 | 308 | 44 |
| 4 | Spinach | 472 | 315 | 157 | 283 | 252 | 31 |
| 5 | Cabbage | 352 | 308 | 44 | 308 | 220 | 88 |
| 6 | Tomato | 220 | 176 | 44 | 176 | 132 | 44 |
| 7 | Zucchini | 264 | 220 | 44 | 220 | 176 | 44 |
| 8 | Cucumber | 176 | 88 | 88 | 132 | 88 | 44 |
| 9 | Potatoes | 132 | 44 | 88 | 88 | 44 | 44 |
| 10 | Carrot | 132 | 44 | 88 | 88 | 44 | 44 |

Based on the total oxalate measurements you conducted in these plants, here’s the ranking from highest to lowest total oxalate content:

1. **Watercress**: Contains the highest content of total oxalates (748mg/ 100g) making it the plant with the most oxalates. It should be consumed with caution, especially for individuals with kidney issues.

**2. Okra**: Comes second in terms of oxalate content (660mg/100g). It contains relatively high levels of oxalates so moderate consumption is recommended.

**3. Parsley:** Contains a significant content of oxalates(528mg/100g). and should be consumed carefully particularly for those prone to kidney stone formation.

**4. Spinach:** Has a high content of oxalates(472mg/100g) and is one of the plants that should be consumed in moderation especially for people with kidney problems.

**5. Cabbage**: Contains a moderate content of oxalates(352mg/100g) compared to the other plants making it suitable for moderate consumption.

**6. Tomatoes**: Contain a moderate content of oxalates(220mg/100g) but they are generally safe for consumption in the diet.

**7. Zucchini:** Has a low level of oxalates content (264mg/100g) compared to other plants making it a good option for regular consumption.

**8. Cucumber:** Contains a very low content of oxalates(176mg/100g) making it safe for daily consumption without concern.

**9. Potatoes:** Contain very low levels of oxalates content (132mg/100g) and are among the safest plants in this regard.

**10. Carrots**: Contain the lowest content of oxalates among this group of plants(132mg/100g) making them an ideal option for regular consumption without worry. It’s important to consume these plants in moderate content particularly those with higher oxalate content such as okra, parsley, and spinach to ensure kidney health as given in table (2). The highest value of total oxalate content was found in watercress (748 mg), and the lowest value in potatoes and carrot (132mg). From table 2 it can be noticed that the boiling of plant will leads to reduce in total oxalate content of all the investigated samples in the present study. Harvesting and post-harvest handling during storage. In Zagazig and in most places tomato and Potatoes are highly consumed vegetables watercress and salad are eaten as raw vegetables and the other analyzed vegetables are eaten as cooked form. On the other hand, the diet with high oxalates content can interfere with calcium absorption in kidney and increase the possibilities to form kidney stones

**2.2-Water samples results:** Water samples were collected from the sources that kidney stone patient's use for drinking in order to analyze the water's properties and its potential impact on their health. The drinking water samples were analyzed for pH, alkalinity, hardness, total dissolved solids (TDS), electrical conductivity (EC), and temperature. Drinking water samples are tap water, ground water and filter water. A water sample was taken from the river in Zagazig city (Bahr Mois River) to analyze it. The water samples were collected systematically from various points to ensure representative analysis.

**Table 3: Analysis of certain parameters of drinking water in collected samples from the patients and non-renal stones diseases**

| sample No, | Location of case patients | Date | Water Temp, C° | EC e (m*S*/cm) | | TDS (mg/L) | | Alkalinity mg/L | | Hardness mg/L | | PH | | Ca mg/L | Mg mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Case patient | Non-patient | Case patient | Non-patient | Case patient | Non-patient | Case patient | Non-patient | Case patient | Non-patient | | |
| 1- | Zagazig (Bahr Mois) | 1/5/2023 | 24 | 560 | 345 | 280 | 292 | 340 | 340 | 260 | 190 | 7.32 | 7.33 | 100 | 160 |
| 2- | Hehia | 1/5/2023 | 24.4 | 160 | 270 | 80 | 180 | 160 | 165 | 160 | 165 | 7.70 | 8.40 | 100 | 60 |
| 3- | Dairb Najm | 22/5/2023 | 28.3 | 360 | 395 | 210 | 190 | 66.7 | 80 | 200 | 150 | 7.48 | 7.89 | 166.6 | 33.4 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4- | Alshabraween village | 24/6/2023 | 30.8 | 350 | 395 | 180 | 170 | 133.3 | 95 | 133.3 | 230 | 8.4 | 8.19 | 120 | 13.3 |
| 5- | Belbeis | 16/7/2023 | 32 | 1220 | 815 | 590 | 480 | 350 | 170 | 350 | 230 | 7.58 | 7.78 | 300 | 50 |
| 6- | Minya Alqamh | 16/10/2023 | | 350 | 370 | 180 | 245 | 280 | 145 | 160 | 165 | 7.6 | 7.81 | 100 | 60 |
| 7- | Abu Kabir city | 16/10/2023 | 28.6 | 370 | 345 | 180 | 150 | 360 | 125 | 200 | 182 | 7.85 | 8.22 | 130 | 70 |
| 8 | Zagazig | 16/10/2023 | 28.8 | 620 | 645 | 310 | 220 | 320 | 170 | 250 | 201 | 7.56 | 7.78 | 200 | 50 |
| 9- | Mit Ghamr | 30/10/2023 | 28.4 | 310 | 340 | 150 | 130 | 460 | 200 | 150 | 117 | 7.8 | 8.22 | 100 | 50 |
| 10- | Belbeis | 30/10/2023 | 28 | 440 | 541 | 220 | 310 | 172 | 95 | 200 | 190 | 7.7 | 7.89 | 100 | 100 |
| 11- | Faqous | 30/10/2023 | 28.2 | 410 | 313 | 200 | 180 | 112 | 65 | 150 | 190 | 7.66 | 7.82 | 100 | 50 |
| 12- | Mashtoul Elsouk | 6/11/2023 | 28.6 | 270 | 210 | 130 | 200 | 120 | 80 | 200 | 182 | 7.5 | 7.60 | 130 | 70 |
| 13- | Belbeis | 6/11/2023 | 28.5 | 570 | 225 | 290 | 245 | 180 | 157 | 150 | 165 | 7.58 | 7.78 | 116.6 | 34 |
| 14- | Kafr Saqr | 6/11/2023 | 28.6 | 810 | 790 | 400 | 422 | 200 | 145 | 350 | 250 | 7.3 | 7.32 | 250 | 100 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15- | Zagazig city | 6/11/2023 | 28.5 | 760 | 708 | 380 | 241 | 800 | 750 | 260 | 180 | 7.38 | 8.02 | 233 | 27 |
| Median | | | | 410 | 447.133 | 252 | 243.66 | 270.26 | 185.4 | 211.5 | 185.8 | 7.627 | 7.87 | 149.7 | 61.8 |

**N.B:** The five samples from Belbeis and the nine samples from Mit Ghamr are under ground water, the high alkalinity and hardness for them are due to the sources of water

**Data Recording and organization**:

All collected data were recorded accurately and organized in a structured table format for further analysis. The table (3) includes the parameters such as PH, temperature, TDS, and a concentration of various contaminates to assess the water quality

**From look at the table (3) it can concluded that:**

The collected water samples underwent a series of analytical tests to determine the concentration of harmful substances including chemicals, heavy metals according to established laboratory methods. The 15 water samples were collected from various regions across Sharqia Governorate to measure several parameters including Water temperature, Total Dissolved Solids (TDS), Electrical Conductivity (EC), pH, Hardness, Alkalinity, Calcium (Ca) concentration, and Magnesium (Mg) concentration. The analysis revealed significant variations in water quality across different areas.

1- **Highest Values**: Moas River water in Zagazig city showed the highest values (280mg/ L,560 m*S*/cm ,260 mg/L ,100 mg/L ,160 mg/L) for TDS, EC, hardness, and mineral concentrations and well water. This indicates that water from these sources is more mineralized and has higher levels of dissolved salts and minerals compared to other sources as in sample number one (1) at Bhar Moas and well water has higher levels as in sample number five (5) from Belbas**.**

**2. Moderate Values**: Tap water showed moderate levels of these parameters as in the sample 6 (180 mg/ L,350 **m***S*/cm,160 mg/L,100 mg/L,60 mg/L) indicating a more balanced water composition possibly due to treatment processes that reduce mineral content as in the samples (7, 9, 12, 13, 14, and 15)

**3. Lowest Values**: Filter water had the lowest values across all parameters reflecting effective filtration and treatment processes that remove most dissolved solids, minerals, and contaminants as in second sample from Hihya (80 mg/ L,160 m*S*/cm,160 mg/L, 100 mg/L,60 mg/L)

**4. High-Measurement Areas:** The areas with the highest measurements were Belbas which showed elevated levels of TDS (590 mg/ L), hardness (350 mg/ L), and mineral concentrations (ca:300 mg/ L, Mg:50 mg/ L) and exceeded the standard limit by WHO (World Health Organization) as given in table (4). This suggests that water quality in these regions may be influenced by local environmental factors, such as water sources or agricultural runoff and most of population in these areas depend on well water. Access to safe drinking water is a basic requirement for healthy life and our study revealed that all the water samples examined were within the permissible limit of the WHO for drinking water quality except ground water.

**Table 4: Limits of drinking water levels for PH, TDS, Hardness, EC, Alkalinity, according to WHO:**

| Parameters | Low level | High level | Standard level |
|---|---|---|---|
| PH | 6.5 drinking water | 8.5 domestic use water | 7.0 pure water |
| TDS ppm | <1500mg /L fresh water | >5000 mg /L saline water | 1500-5000 mg /L brackish water |
| EC | 5 $x10^3$ (ms/cm) sea water | $5.5x10^{-6}$ (ms/cm) Ultra-pure water | 0.005-0.05 (ms/cm) drinking water |

| | | | | |
|---|---|---|---|---|
| Hardness | ppm | <150mg /L hardness water | >500mg/L is safe | 300mg /L |
| Alkalinity | ppm | 200 ppm | 600 ppm | 400 ppm |
| Calcium Concentration | ppm | 10 mg/L | 200-300 mg/L | 100-200 mg/L |

Monitoring these values and ensuring they comply with WHO standards is critical to ensuring water quality and safety for human consumption. So, the monitoring values such as pH, Total Dissolved Solids (TDS), Electrical Conductivity (EC),

Hardness, and Alkalinity is crucial for assessing water quality and ensuring its suitability for human consumption. According to the World Health Organization (WHO). And through research and study it was found that Calcium is one of the essential nutrients present in drinking water. To determine its concentration Atomic Absorption Spectroscopy (AAS) is employed. This technique involves measuring the absorbance of specific wavelengths of light by calcium atoms in a flame, allowing for precise quantification. Studies have successfully utilized AAS to analyze calcium levels in various water samples including tap water and well water.

**Table5. Measurement of Ca concentration in drinking water**

| Samples No, | Region | Date | Source | Ca concentration mg/L |
|---|---|---|---|---|
| 1- | Hihya (Tayba village) | 1/2/2024 | well | 74.2-80.3 |
| **2-** | Zagazig | 1/2/2024 | tap | 43.5 |
| **3-** | Zagazig | 1/2/2024 | tap | 46.5 |
| **4-** | Belbas | 15/2/2024 | well | 80 |
| **5-** | Belbas | 15/2/2024 | well | 55 |
| **6-** | Belbas | 15/2//2024 | tap | 48.6-51 |
| **7-** | Faqose (Al-Samaana) | 27/2/2024 | well | 51-54.5 |
| **8-** | Faqose | 27/2/2024 | tap | 50 |
| **9-** | Faqose | 27/2/2024 | tap | 45 |
| **10-** | Bahr Moas | 27/2/2024 | river | 70 |

**N.B:** The sample number four the highest concentration of calcium was recorded at station No,4 due to the mixing between well water and domestic sewage

**Calcium Concentration in Drinking Water Samples for Kidney Stone Patients: -**

**Table 5 shows that calcium concentration ranged from**

**1. Samples from Wells in Villages of Hihya City (Al-Tayba Village):**

Results: Ranged from 74.2 mg/L to 80.3 mg/L. The results show moderate to high calcium concentrations in well water in Al-Tayba. For individuals with kidney stones this concentration could be a concern as high calcium levels in drinking water may contribute to the formation of kidney stones over time.

**AL-Samaana Village**: The results of Ca ranged from 51mg/L to 54.5 mg/L as indicated in table (5).

These values indicate a higher concentration of calcium compared to other areas. For kidney stone patients the elevated calcium levels may increase the risk of further stone formation as calcium can accumulate in the kidneys and contribute to stone formation.

**2. Samples from Tap Water in Cities (Zagazig, Belbeis, Faqous)**

**Zagazig:** The results ranged from 43.5 mg/L to 46.5 mg/L as given in table (5).

The calcium concentration in Zagazig's tap water is moderate. Although these values are within the acceptable range for drinking water individuals with kidney stones should be cautious. Over time, regular consumption of water with higher calcium levels may contribute to kidney stone formation.

**Belbeis:** The results ranged from 48.6 mg/L to 51.3 mg/L as shown in table (5).

The results show relatively high calcium concentrations. For kidney stone patients these higher levels might pose a risk by contributing to the formation of new stones as calcium accumulation is a common factor in stone development.

**3. Samples from rivers (Bahr Moas, Canals): -**

**Bahr Moas River (Zagazig and Faqous areas):** The results varied from 54.0 mg/L to70 mg/L as given in table (5). The results show relatively high calcium concentrations. For kidney stone patients these higher levels might pose a risk by contributing to the formation of new stones. **Permissible limit mg/L: low below200 mg/L as given in table (4).**

**Source Classification**:

**Well:** Water sourced from underground wells.

**Tap**: Water obtained from public water supply taps samples from the sources that kidney stone patient's use for drinking.

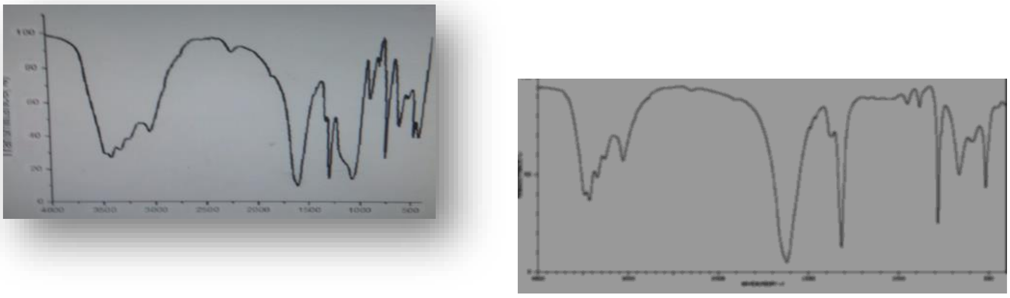
**Raw water**: Water sourced from rivers.

**Standard Permissible Limit for Calcium Concentration in Drinking Water**: According to the World Health Organization (WHO), there is no health-based guideline value for calcium in drinking water. However, water hardness, which is influenced by calcium and magnesium concentrations typically ranges from 10 to 500 mg of calcium carbonate per liter. Potential Impact on Kidney Stone Formation: The calcium concentrations measured in this study are all below the 200 mg/L threshold. Therefore, they are unlikely to have a significant impact on kidney stone formation.

**Health Implications:** While calcium is essential for human health excessive concentrations in drinking water can contribute to water hardness which has been associated with certain health benefits such as a possible protective effect against cardiovascular diseases. **Preventive Measures:** To mitigate potential adverse effects of hard water individuals may consider using water softening methods or consuming adequate dietary calcium from other sources.

**3 – Stones Samples Results:** The cases selected by the Urology Department at Zagazig University Hospital underwent surgical removal of renal stones after which the stone samples were collected and transferred to the analytical laboratory for composition analysis. The samples were analyzed using a Bruker Tensor 27 Fourier Transform Infrared (FT-IR) Spectrometer, manufactured by Bruker Optik GmbH, Ettlinger, Germany with a production year of 2010. This instrument provides high spectral resolution and stability making it suitable for accurate identification of kidney stone components. For sample preparation, each stone was finely ground and mixed with spectroscopic-grade potassium bromide (KBr) to form a transparent pellet. The use of KBr enhances the transmission of infrared radiation and allows precise detection of characteristic absorption peaks corresponding to major stone constituents such as calcium oxalate, uric acid, carbonate apatite, and struvite. This analytical approach ensures reliable determination of stone composition which is essential for accurate diagnosis and clinical decision-making. For reference, the role of KBr in IR spectroscopy and its importance in solid-state analysis are well documented in the work of Smith and Dent (2005), "Modern Raman Spectroscopy: A Practical Approach," John Wiley & Sons, which discusses standard sample preparation techniques for infrared and Raman analysis.

**IR Spectra of Calcium Oxalate Stones**

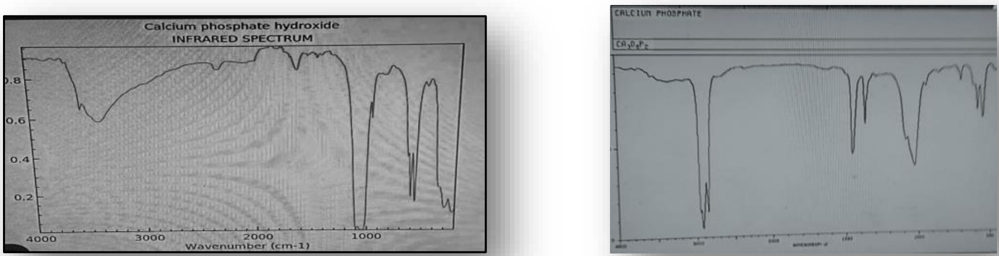


**(A)Standard spectrum** **(B) Sample spectrum**

**Fig (10) IR Spectra of Calcium Oxalate Stones (c=o at1600-1650cm$^{-1}$)**

**Dark brown or yellow stones: Typically associated with calcium oxalate stone**

**IR Spectra of Calcium Phosphate Stones**

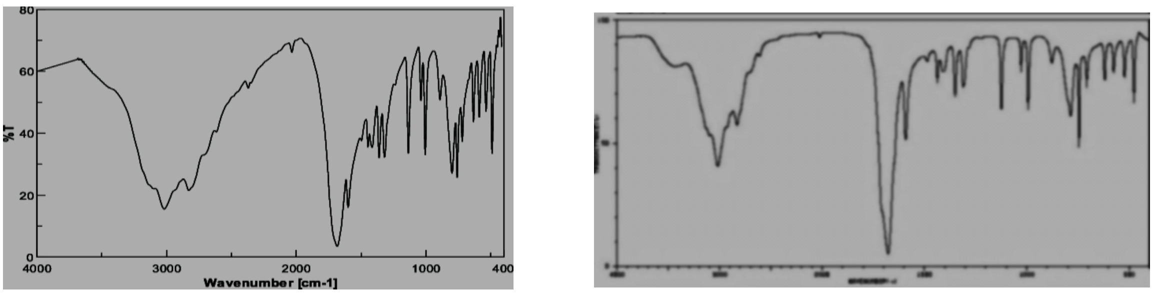



**(A) Standard spectrum** **(B) Sample spectrum**

**Fig (11) IR Spectra of Calcium Phosphate Stones (P-O at 900-1100cm$^{-1}$)**

**White or gray stones: Often indicates calcium phosphate or struvite stones**

**IR Spectra of Uric Acid Stones**




**(A) Standard spectrum** **(B) Sample spectrum**

**Fig (12) IR Spectra of Uric Acid Stones (C=O at 1700cm$^{-1}$)**

**Light yellow stones: Associated with uric acid stones**

The resulting spectra are a key to understanding the chemical composition of the stone. These results involve understanding the peaks observed in the spectrum each corresponding to a specific vibrational mode of molecules. Fourier Transform Infrared (FTIR) spectroscopy analysis was performed on various types of kidney stone samples using the potassium bromide (KBr) pellet method to identify their chemical composition and functional groups. The spectra revealed the presence of characteristic functional groups corresponding to the major constituents typically found in kidney stones such as calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) and uric acid. Specific absorption bands observed in the IR spectra were indicative of key functional groups such as carbonyl (C=O), hydroxyl (O–H) and phosphate ($PO_4^{3-}$) providing qualitative insights into the stone. The FTIR-KBr technique proved to be a reliable and non-destructive method for differentiating between the chemical types of kidney stones thereby aiding in better understanding of their etiology and guiding clinical management strategies for urolithiasis [11, 12, 13, 14, and 15].

**There are different IR Spectra of stones samples for patient cases as following: -**

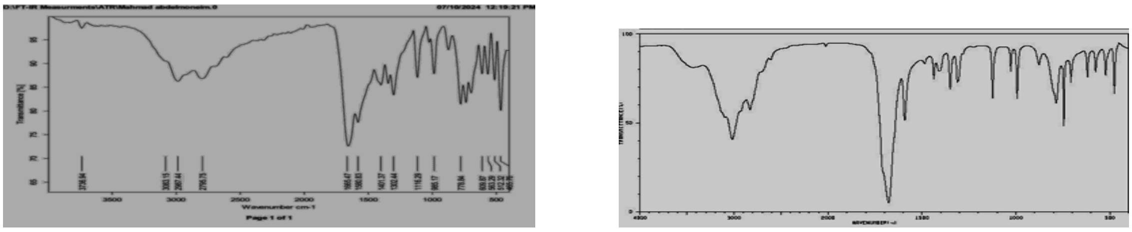


**Fig (13) IR Spectra of stone sample (Uric Acid) (C=O at 1700cm$^{-1}$)**

**Different IR Spectra of stone sample: -**

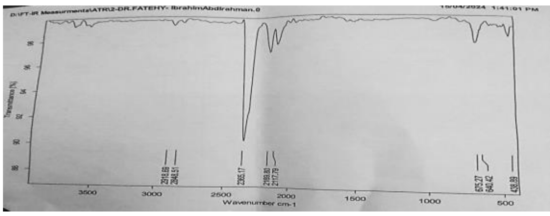


**Fig (14) IR Spectra of stone sample**

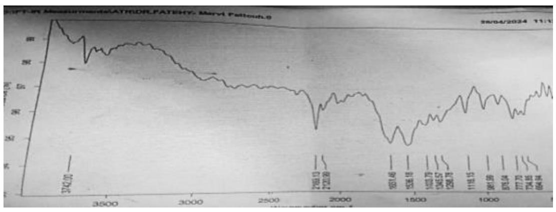


**Fig (15) IR Spectra of stone sample**

**Urine samples from kidney stone patients were analyzed as follows:** A total of 50 urine samples were collected from patients diagnosed with nephrolithiasis in Zagazig University Hospital in Sharqia. The biochemical analysis of these samples demonstrated a range of urinary abnormalities commonly associated with stone formation as following in table 6:

**Table6.Urinalysis Results of Some Samples from Kidney Stone Patients**

| Parameter | Sample1 | Sample2 | Sample3 | Sample4 | Sample5 | Normal ranges |
|---|---|---|---|---|---|---|
| Color | Yellow | Dark yellow | Yellow | Amber | Pale yellow | Pale yellow to amber |
| Appearance | Slightly cloudy | Cloudy | Clear | Turbid | Slightly cloudy | Clear |
| PH | 5.8 | 6.0 | 5.5 | 6.2 | 5.6 | 4.6-8.0 |
| Specific Gravity | 1.030 | 1.028 | 1.032 | 1.029 | 1.027 | 1.005-1.030 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Protein | Negative | Trace | Negative | +1 | Negative | Negative - Trace |
| Blood | Positive | Positive | Trace | Positive | Trace | Negative |
| Calcium(mg/dL) | 12.5 | 11.2 | 13.8 | 14.0 | 10.5 | 2.5-7.5 mg/dL (random sample) or <250mg/24h |
| Oxalate(mg/24h) | 45 | 52 | 47 | 60 | 42 | 4-31mg/24h(adults) |
| Crystals | Calcium Oxalate | Calcium Oxalate | Uric acid | Calcium Oxalate | Uric acid | None or occasional |
| RBCs /HPF | 10-15 | 8-12 | 4-6 | 12-18 | 6-10 | 0-3 / HPF |
| Leukocytes/ HPF | 3-5 | 5-7 | 1-2 | 6-8 | 0-1 | 0-5 / HPF |

N.B: (RBCs / HPF) Red Blood Cells per High Power Field, (Leukocytes / HPF) White Blood Cells per High Power Field.

**From table 6:** Urinalysis Interpretation and Clinical Commentary the urinalysis data presented in the table 6 demonstrate consistent patterns commonly associated with nephrolithiasis (kidney stone disease). All five patients exhibit abnormalities that correlate with their clinical diagnosis of renal calculi.

**Urinary Calcium and Oxalate**: Urinary calcium levels in all patients exceed the upper limit of the normal range with values ranging from 10.5 to 14.0 mg/dL (reference: 2.5–7.5 mg/dL in random samples or <250 mg/24h). Similarly, urinary oxalate excretion is elevated in all samples (42–60 mg/24h vs. a normal range of 4–31 mg/24h), supporting the likelihood of calcium oxalate stone formation in most cases. These two markers are among the most significant risk factors for stone development.

**Crystalluria and Stone Type Indication:** The presence of calcium oxalate and uric acid crystals in the urine supports the biochemical analysis indicating stone composition. Calcium oxalate crystals are found in patients 1, 2, and 4 while 3 and 5 presented uric acid crystals suggesting possible mixed stone formation or an underlying metabolic predisposition.

**Hematuria and Inflammation:** All patients show either positive blood test results or elevated RBC counts (up to 18/HPF), indicating microscopic hematuria. This is a common finding in stone formers due to mechanical irritation caused by stones passing through the urinary tract.

**Leukocyturia and Possible Infection:** Mild leukocyturia was detected in several patients especially P002 and P004(patients' cases), raising the possibility of secondary urinary tract infections (UTIs) which may further promote stone formation or worsen clinical symptoms.

**Urine pH and Specific Gravity:** The urinary pH in all patients is within the slightly acidic range (5.5–6.2), which favors the formation of calcium oxalate and uric acid stones. Specific gravity values are at the higher end of the normal range indicating concentrated urine a known risk factor for crystallization and stone formation.

**Notes:**

**PH**: Urinary pH tends to be slightly acidic in calcium oxalate stones.

**Specific Gravity:** Elevated levels reflect concentrated urine a common risk factor for stone formation.

**Protein:** Usually negative or trace persistent proteinuria may indicate additional renal pathology.

**Blood:** Hematuria (microscopic or gross) is common in kidney stone patients due to mucosal irritation.

**Calcium & Oxalate:** Elevated urinary calcium and oxalate are the most common metabolic abnormalities in calcium oxalate stone formers.

**Crystals**: Presence of calcium oxalate or uric acid crystals is typical and diagnostic.

**RBCs & Leukocytes per HPF (High Power Field**): Indicates microscopic hematuria and possible secondary infection or inflammation.

These urinalysis profiles strongly support the clinical diagnosis of nephrolithiasis (kidney stone disease). The elevated levels of calcium, oxalate, and urinary crystals combined with microscopic hematuria and mildly increased leukocytes suggest active stone formation or passage. Management should include metabolic evaluation, hydration, dietary modifications, and possibly pharmacologic intervention to reduce recurrence.

**Analysis of Complete Blood Count (CBC) Samples in Patients with Kidney Stones:** Blood samples were collected from a total of 50 patients diagnosed with kidney stones. Complete Blood Count (CBC) analysis was performed to identify any hematological patterns or abnormalities associated with different types of kidney stones as following in table 7

**Table7. Complete Blood Count (CBC) Samples in Patients with Kidney Stones**:

| Parameter | P1 | P2 | P3 | P4 | P5 | Normal range |
|---|---|---|---|---|---|---|
| Hemoglobin (g/dL) | 11.2 | 12.8 | 13.5 | 10.9 | 12.1 | 12.0-16.0(F)/ 13.5-17.5(M) |
| Hematocrit (%) | 34.1 | 38.5 | 40.2 | 32.8 | 36.0 | 36-46 (F)/41-53(M) |
| WBC ($\times 10^3$/ µL) | 11.8 | 9.4 | 8.6 | 13.2 | 10.5 | 4.0-10.0 |
| Neutrophils (%) | 72 | 68 | 65 | 78 | 70 | 40 -70 |
| Lymphocytes (%) | 20 | 24 | 28 | 15 | 22 | 20-40 |
| Platelets ($\times 10^3$/ µL) | 280 | 320 | 310 | 295 | 305 | 150-400 |
| $RBC_s$ count ($\times 10^6$/ µL) | 4.1 | 4.5 | 4.7 | 3.9 | 4.3 | 4.2-5.4(F)/4.7-6.1(M) |

**N.B: P1, P2, P3, P4, P5 are patients' cases in the present study.**

From look at table 7 it can be noticed that

**Clinical Commentary from Urology Department, Zagazig Hospital:**

**Mild Anemia**: Patients P1 and P4 show slightly reduced hemoglobin and hematocrit levels likely due to chronic hematuria associated Urology Department, Zagazig Hospital with stone passage.

**Leukocytosis and Neutrophilia**: Elevated total WBCs and neutrophil counts in patients P1, P4, and P5 may indicate urinary tract infections or inflammation, which are common complications in stone-formers.

**Stable Platelet Counts:** All patients have normal platelet counts which rules out any significant coagulopathy.

**RBC Count:** Slight reductions in RBCs in P4 may correlate with blood loss through hematuria.

Based on the chemical composition of the stones the distribution among the patients often associated with metabolic

conditions such as hyperuricemia or low urinary pH.5% to 10% presented with calcium phosphate stones which are sometimes related to renal tubular acidosis or urinary tract infections. Preliminary observations indicated a higher prevalence of stone formation among smokers suggesting a potential link between tobacco exposure and the risk of nephrolithiasis. Additionally, among the younger patients a notable proportion reported having fathers who were smokers which may imply a correlation between passive smoking during early life and the early onset of kidney stone formation. The CBC parameters showed variable trends across stone types. Nevertheless, inflammatory markers such as elevated white blood cell (WBC) counts and neutrophil to lymphocyte ratio (NLR) appeared slightly increased in patients with active stone episodes or recent renal colic particularly among calcium oxalate stone formers. These findings highlight the importance of considering lifestyle and environmental factors such as smoking in addition to metabolic and dietary contributors in the pathogenesis of kidney stones which is also shown in the following table8

**Table 8. Characteristics of the renal stones' patients (n=50):**

| Character | | Value |
|---|---|---|
| Age | | 7-67 |
| Gender | Male | 35 |
| | Female | 15 |
| Residence | Urban | 30 |
| | Rural | 20 |
| Smoking (%) | Smoker | 40-50% |
| | Non-Smoker | 50-60% |
| Average size stone | | 2or3ml to 3cm -5cm |
| Stone location | | Left and right kidney, ureter and urinary bladder |
| Major constituent of stone (%) | | |
| Calcium oxalate | | ~60 to70 |
| Calcium phosphate | | ~5 to 10 |
| Uric acid | | ~10 to 20 |
| Symptoms | | Flank and back pain, hematuria, nocturia, burning sensation during urine pas |

From the analysis of the tables 2, 3, 4 and 5, several factors that influence the formation of kidney stones in patients can be observed. These factors include the type of water consumed (well water, tap water, or filtered water), poor nutrition, and smoking. The most likely and significant cause of stone formation appears to be smoking. Additionally, inadequate water intake plays a critical role in influencing kidney health.

**Factors which lead to stones formation in kidney:**

**1. Type of Water**

**Well Water**: Well water often contains higher levels of minerals such as calcium and magnesium. These minerals are associated with the formation of calcium-based stones particularly calcium oxalate and calcium phosphate stones. If the well water is not properly treated or contains impurities, the risk of stone formation increases significantly.

**Tap Water**: Tap water can vary in its mineral content depending on the treatment processes in urban and rural areas. While it generally contains fewer minerals compared to well water the mineral concentration can still be sufficient to contribute to stone formation in susceptible individuals.

**Treated Water:** Filtered water which undergoes purification processes, generally contains lower levels of minerals. While this can reduce the risk of stone formation it is important to ensure that filtered water still contains essential minerals like magnesium and calcium in appropriate amounts for overall health.

**2. Poor Nutrition:** Poor nutrition is a significant contributing factor to the stone formation. Unbalanced diets particularly those rich in sodium, animal protein, and foods high in oxalates (such as spinach and okra) can lead to the accumulation of stone forming substances like calcium and oxalate in the urine. Furthermore, insufficient intake of fruits, vegetables, and healthy water can lead to dehydration which increases the likelihood of stone formation.

**3. Smoking:** The table indicates that most patients with kidney and bladder stones are smokers suggesting that smoking plays a major role in stone formation. Smoking negatively impacts kidney function by decreasing renal blood flow and causing dehydration. Moreover, the toxic chemicals in tobacco smoke can disrupt normal urinary tract function leading to an increased risk of stone formation. Smoking may also increase the excretion of substances such as calcium and oxalate. Furthermore, contributing to the formation of stones. Research and study have shown that in young cases of childhood, the father was a smoker in addition to the previous reasons.

**4. Inadequate Water Intake:** Inadequate water intake is a primary risk factor for stone formation. When individuals fail to drink sufficient amounts of water (about 2.5L) it leads to concentrated urine, which in turn increases the likelihood of stone-forming minerals (like calcium, oxalates, and uric acid) crystallizing and forming stones. Hydration is crucial for diluting these substances in the urine, preventing stone formation, and maintaining overall kidney and urinary tract health. (Original Article-Endourology/Urolithiasis2018)

**Impact of These Factors on Health:** The combination of these factors poor water quality, inadequate hydration, poor nutrition, and smoking can significantly increase the risk of developing kidney and bladder stones leading to adverse health outcomes such as severe pain, recurrent urinary tract infections and potential kidney damage. Therefore, it is essential to address these contributing factors to prevent stone formation and maintain renal health. This can be achieved by improving water quality promoting sufficient fluid intake adopting a balanced diet and avoiding smoking. Such measures are vital in preventing the formation of kidney and bladder stones and ensuring optimal urinary tract health [16,17,18,19].

## Conclusion

The water quality in Sharqia Governorate varies significantly based on location and source, with well and river water having the highest concentrations of dissolved solids and minerals while tap water and filtered water show lower concentrations. Kidney stones are solid masses formed from substances like calcium oxalate and uric acid that are normally present in urine. The ground water dietary habits and excessive consumption of food containing large amounts of oxalates significantly influence the formation and recurrence of these stones.

**Impact of Water Quality**: The mineral content of drinking water, particularly its hardness determined by calcium and magnesium with high levels has been linked to kidney stone risk. Some studies suggest that water with high total dissolved solids (TDS) or elevated calcium and magnesium concentrations may increase the likelihood of stone formation, especially in individuals predisposed to nephrolithiasis. However, other research emphasizes that the quantity of water consumed is more critical than its quality in preventing kidney stones.

**Influence of food:** Food plays a pivotal role in kidney stone prevention. Adequate hydration is essential drinking about 2.5 L of water daily helps dilute urine, reducing stone formation risk. Consuming a balanced diet rich in fruits and vegetables provides necessary nutrients and helps prevent stone formation. Limiting intake of sodium, oxalate-rich foods and animal proteins can also reduce risk.

**Influence of Smoking:** Recent studies have established a significant association between smoking and an increased risk of kidney stone formation. For instance, research presented at the American Society of Nephrology's Kidney Week in November 2023 highlighted that cigarette smoking is linked to a heightened risk of developing kidney stones [20,21].

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