



Effect of aquagenic and lithogenic factors on nephro, uretro and urolithiasis

Kumar Ajay^{1*}, Singh Neetu¹, Srivastava Ankita¹ and Swaroop Kavita²

¹Department of Biotechnology, Mewar Institute of Management, Sec-4C, Vasundhra, Ghaziabad, U.P-201012, India.

²Department of Biotechnology, Mewar University, Rajasthan, India.

Abstract

Our study aims to explore different lithogenic and aquagenic factors which lead to the formation of renal calculi. To interpret this, a standard questionnaire is prepared based on the available review data which includes all the variable dietary information's and other physiological parameters responsible for renal calculi. A study sample size of individuals (n=500) varies 19-65 years of age belongs to Delhi and NCR region. Of the 500 individuals more than 35 percent of individuals' shows routine consumption of vegetables items includes eggplant, okra and ginger. 77 percent of the total individuals' showed routine consumption of seasonal vegetables includes radish, carrot and spinach. 85 percent of the total shows routine uptake of whole wheat, grains, gram, black-pepper, soybean and nuts. 82 percent of the total individuals shows regular seasonal uptake of grapes while 35 percent of total shows routine uptake of strawberry and kiwi. Among beverage consumption 92 percent individuals takes tea, coffee, carbonated drink cola in routine while 46 percent takes Juice- apple, Grape, orange in routine and 75 percent takes whisky, beer, wine and other alcoholic beverages. In order to study the effect of potable water on the formation of renal calculi, water samples were collected from 20 different residential places (RPs) of Delhi- NCR and characterize for pH, calcium, magnesium and sodium.

Keywords: Renal calculi and Potable water.

INTRODUCTION

The kidney acts as a filter for blood, removing waste products from the body and making urine. It also helps in regulating electrolyte levels that are important for body functions. Urine drains out from the kidney through a thin walled narrow tube called the ureter into the bladder. When the bladder fills and there is an urge to urinate, the bladder empties to the outside through the urethra, a much wider tube than the ureter. In some peoples, chemicals crystallize in the urine and mark the beginning of a kidney stone. These stones are very tiny when they form, smaller than a grain of sand, but gradually can grow over time to 1/10 of an inch or larger. Urolithiasis is the term that refers to the presence of stones in the urinary tract, while nephrolithiasis refers to kidney stones a, while nephrolithiasis refers to kidney stones and ureterolithiasis refers to stones lodged in the ureter. The size of the stone doesn't matter as much as where it is located and whether it obstructs or prevents urine from draining. When the stone sits in the kidney, it rarely causes problems, but when it falls into the ureter, it acts like a dam. As the kidney continues to function and make urine, pressure builds up behind the stone and causes the kidney to swell. This pressure is what causes the pain of a kidney stone, but it also helps push the stone along the course of the ureter. When the stone enters the bladder, the obstruction in the ureter is relieved and the symptoms of a kidney stone are resolved.^[1] Besides adults even children are experiencing

the problem of renal stone formation which is an all-time high.^[2] Kidney stones cause's considerable suffering and have a substantial economic impact.^[3] As per the review of literature, among common important dietary factors of renal calculi includes Citrate, Sodium(Na), Calcium(Ca), Vitamin-C, Animal proteins, Oxalate, etc.^[4] usually present in common foods, vegetables and fruits like tomato, spinach, grapes, nuts & grains, salt besides other food items and supplements like orange juice, tea, coffee, and Vitamin-C, Calcium(Ca) supplements, etc.^[5] Human body contain oxalates, and our cells routinely convert other substances into oxalates. For example, vitamin C is one of the substances that our cells routinely convert into oxalates. In addition to the oxalates that are made inside of our body, oxalates can arrive at our body from the outside, from certain foods that contain them. For patients suffering from renal calculi not more than 50 mg/d is recommended. The questionnaire prepared was asked to the patients of Delhi-NCR coming at various city hospitals and other charitable treatment sites like Balaji Mandir, Vivek-vihar. 500 people were questioned randomly in order to make a study size a heterogeneous one. Based upon the questionnaire results, 20 sampling stations were selected for Aquagenic analysis of water samples.

REVIEW OF LITERATURE

Renal Calculi and their types

Renal calculi are inorganic crystalline aggregates enmeshed in about 5.0% organic matrix. This disease has tormented humans since the earliest records of civilization. About 10.0% of men and 3.0% of women have a stone during their adult lives.^[6] In majority of kidney stones, calcium oxalate (CaOx) is the main constituent (80.0%) and calcium phosphate, Ca₃(PO₄)₂ is present in amounts ranging from 1.0% to 10.0% ; 10.0% of struvite, 9.0% uric acid and the remaining 1.0% are composed of cystine or drug-related stones.

*Corresponding Author

Kumar Ajay
Department of Biotechnology, Mewar Institute of Management, Sec-4C,
Vasundhra, Ghaziabad, U.P-201012, India.

Email: ajaykmr1986@gmail.com

The prevalence of calcium oxalate stones has been constantly increasing during past fifty years in industrialized as well as in developing countries and varies depending on race, sex and geographic locations. Basically the renal stones can be divided into two major groups' i.e. primary stones and secondary stones. [7]

Lithogenic factors responsible for Renal Calculi

Among important lithogenic factors responsible for renal calculi includes Calcium, Oxalate, Phosphate, Uric acid, Magnesium ammonium phosphate and amino acid cystine via different routes. [15] Dietary practices that may increase an individual's risk of forming kidney stones include a high intake of animal protein, a high-salt diet, excessive sugar consumption, excessive vitamin D supplementation, and possible excessive intake of oxalate-containing foods such as spinach. Interestingly, low levels of dietary calcium intake may alter the calcium-oxalate balance and result in the increased excretion of oxalate and a propensity to form oxalate stones. [16] Metabolic factors involved in stone formation include hypercalciuria (found in 50% of patients and its most common cause is increased intestinal calcium absorption), hypocitraturia (due to renal disease), hyperuricosuria, hyperoxalaturia, cystinuria and infection. [17, 18] In the study of 78,293 women from the Women's Health Initiative Observational Study, 2.5% reported an incidence of kidney stone formation. Among these women the risk of kidney stones was increased by 11 – 61% with higher dietary sodium intake, with the most pronounced effect in women with the highest intake. [19] Increased ingestion of animal protein produces a significant increase in the urinary excretion of calcium, oxalate and uric acid. Oral carbohydrate loading increases urinary calcium and magnesium excretion. A rational, though not of proven efficacy, dietary approach to urolithiasis therapy includes restriction of animal protein, avoidance of excess oxalate ingestion, a normal calcium intake, and water intake sufficient to generate 2 litres of urine per day. So it seems that protein ingestion, carbohydrate ingestion and foods rich in oxalate all contribute to kidney stones. [20] It's definitely advisable to reduce sodium levels, which means the refined salt that is added to processed foods and regular table salt which contains anti-caking agents and in some countries, fluoride and sugar. The average intake of sodium in America is 3.7 to 5 grams per day, considerably higher than the recommended maximum of 2.3 grams. And the more excess salt consumed, the higher the risk. Maintain a healthy weight, thereby avoiding another risk factor: high Body Mass Index (BMI). Uric acid stones, specifically, have been tied to the increase of obesity and metabolic syndrome. Achieving and maintaining a healthy weight can ward off a majority of diseases, including cancer, heart disease, diabetes, etc. [21]

Aquagenic factors responsible for Renal Calculi

The hardness of water is due to the presence of carbonate & sulphate salts of calcium and magnesium. More than 3/4th of kidney stones are generally composed of calcium salt and usually occur as calcium oxalate and less commonly as calcium phosphate. Stones form in urine that is supersaturated and this saturation is dependent on chemical free ion activity, which makes the urine become under-saturated. In this situation the stone will not grow and may even dissolve. Increased urinary ion excretion and decreased urine volume will both increase free ion activity and favour stone formation and growth. The impact of water hardness on urinary

stone formation remains unclear, despite a weak correlation between water hardness and urinary calcium, magnesium, and citrate excretion. Several studies have shown no association between water hardness and the incidence of urinary stone formation. A correlation between water hardness and urinary calcium, citrate and magnesium levels has been observed although the significance of this is not known. Some studies suggest that in the preventive approach to calcium nephrolithiasis, intake of soft water is preferable to hard water, since it is associated with a lower risk for recurrence of calcium stones. [22]

OVERVIEW OF RESEARCH DESIGN

The present study was conducted at Department of Biotechnology, Mewar Institute, Vasundhara, Ghaziabad. The study population included patients diagnosed with renal stones. A sample size of individual (n=500) with renal stones in the age between 19-65 years belongs to Delhi-NCR region were included for study. The study consisted of 6 months period (Jan 2012 to June 2012). Questionnaires were completed by interviewing patients covering the information pertaining to their age, sex, habits and health status.

The sampling stations were Delhi (North & East and West & South) and NCR (Ghaziabad, Noida, and Gurgaon extension) selected on the basis of Questionnaire survey. Each Location is divided into four different sampling stations based on Questionnaire survey and review of Literature. From each sampling station, three water samples were collected which includes potable supply Tap water (Municipal supply water), Filtered water (AquaGuard/ Reverse Osmosis systems etc.) and Boring (Submersible/ hand-pump) water. Mineral water as a fourth sample type was obtained from Bisleri outlet available in 2 litre plastic packaged bottle. Water samples from (excluding Mineral water) the sampling stations were collected in 2 litre good quality plastic bottles in duplicate. Thus the total sample size equals to n=124. For the purpose of aquagenic analysis of preserved potable water samples, chemical parameters includes pH, Calcium, Sodium, Magnesium, were analysed using standard methods of estimation as outline in APHA, 2004.

To start with, the beginning of a questionnaire must ask for personal information about the user such as name, address, phone number etc. In some cases, age and other private information can also be required. The next part of the questionnaire must be the part where questions related to the topic are asked point wise with enough detailing of the question and its meaning. The general dictum for any questionnaire is that the questions should be short and direct. In this manner the concerned persons who write the answers find it convenient to reply as there is less space for misinterpretation. It is also important to see that there are no typos and/or grammatical errors in the questions.

LITHOGENIC ANALYSIS OF DIFFERENT WATER SAMPLES:

Whenever a disease appears, it is mandatory to analyze the diet which the patient is taking so as to determine the factors which are contributing towards it. In order to analyze the main lithogenic factors causing renal calculi in Delhi-NCR individuals a questionnaire regarding the dietary habits of patients was done. From the survey undertaken among randomly selected 500 renal calculi patients the important factor which came up was that about 83% patients were in age-group of 22-40. In the patient population about 80% were males suffering from renal calculi. It was observed

that the patients were having the kidney stones repeatedly. In the research questionnaire the question were simply related to their name, place of residence, age, sex and day to day dietary habits. According to Indian diet prominent drinks and eatables have been considered. According to Harvey (2010), edible Ca extracts, citrus juices and oxalate vegetables promotes renal calculi. As per the literature review and preliminary survey report it was decided to survey the following elements with the help of self-designed questionnaire exclusively based on preliminary survey report:

Table 1. Represents dietary intake ratios among randomly selected individuals of Delhi -NCR suffering from Renal Calculi

S.No.	DIET	PERCENTAGE
1.	Tea	95%
2.	Coffee	86%
3.	Cold-drinks	85%
4.	Juices	79%
5.	Alcohol	75%
6.	Tomato	89%
7.	Spinach	62%
8.	Carrot	64%
9.	Radish	70%
10.	Okra	81%
11.	Brinjal	76%
12.	Potato	98%
13.	Ginger	97%
14.	Strawberry	33%
15.	Grapes	86%
16.	Kiwi	30%
17.	Wheat grain	99%
18.	Wheat bran	46%
19.	Soybean	85%
20.	Black pepper	82%
21.	Almond	90%
22.	Cashewnut	86%
23.	Peanut	90%
24.	Beans	64%
25.	Sesame	45%
26.	Oats	32%
27.	Banana	94%
28.	Salt	100%
29.	Milk	78%
30.	Fruit cake	73%
31.	Vitamin D supplements	30%
32.	Calcium supplements	12%
33.	Vitamin C supplements	20%

Above surveyed data shows that the observed, that North Indian diet contains primarily of foods which are high in oxalate, contains purines, high in protein (pulses, beans, soybean, milk, peanuts) and high spices. Indian Tea and coffee as well as alcohol are all dehydrating in nature which leads to concentration of stone elements instead of flushing them out. All these factors either solely and/or in effects with other factors contribute towards building of varied types of renal calculi.

AQUAGENIC ANALYSIS

According to the second objective of our present investigation water samples from different regions were collected and analysed for pH, Ca, Mg and Na in order to assess its suitability for drinking purpose. The initial physiochemical analysis of municipal supply of tap water reflects a requirement of treatment

due to higher concentration of above said parameters in some collected water samples. On the basis of preliminary data, study was further elaborated with some additional potable water sample types which include tap water, filter water and bouring water. The detail of pH, Calcium (Ca), Magnesium (Mg) and Sodium (Na) and Mg/Ca ratio of tap water, bouring water and filter water samples has been recorded, but due to journal limitations we aoly represent coorelation matrix of the collected data. Any change in a single parameter in drinking water is supposed to, directly or indirectly may reflects a series of changes in biological set up of an organism gradually. According to Vollenweider (1968); the chemical changes in a water body are a reflection of the geology and cultural activity in its catchment. The fluctuation in different parameters of water samples primarily is determined by nature of any water body depends upon the interaction of the biotype and biocene. Any change in their environment may be broadly grouped under physical, chemical and biological categories. The different parameters like Ca, Mg etc. were investigated in the present aquagenic study and the data is recorded in tabular form to interpret and correlate the interrelationships between different parameters using correlation matrix and discussed accordingly.

Table 2. Co-relation matrix for tap water.

	pH	Calcium	Magnesium	Sodium
pH	1			
Calcium	0.1668	1		
Magnesium	0.0774 ^{NS}	0.149 ^{NS}	1	
Sodium	0.132 ^{NS}	0.3477	0.236	1

*Significant at 0.01level (*p <0.01), NS = Non- significant.

Above correlation matrix was analysed at 1% level of significance (*p <0.01). From the above co-relation matrix it is inferred that a noticeable significant co-relation exists between Calcium and pH of the water samples taken. However, weak correlation was observed between sodium–pH and sodium-calcium.

Table 3. Co-relation matrix for boaring water

	pH	Calcium	Magnesium	Sodium
pH	1			
Calcium	*0.608	1		
Magnesium	*0.469	*0.394	1	
Sodium	*0.453	*0.684	0.418	1

*Significant at 0.01level (*p <0.01), NS = Non- significant.

Above correlation matrix was analysed at 1% level of significance (*p <0.01). From the above co-relation matrix it is inferred that a considerable significant co-relation exists between pH-calcium and sodium–calcium of the water samples taken. However, weak correlation was observed between sodium–pH and sodium-calcium.

Table 4. Co-relation matrix for Filter water

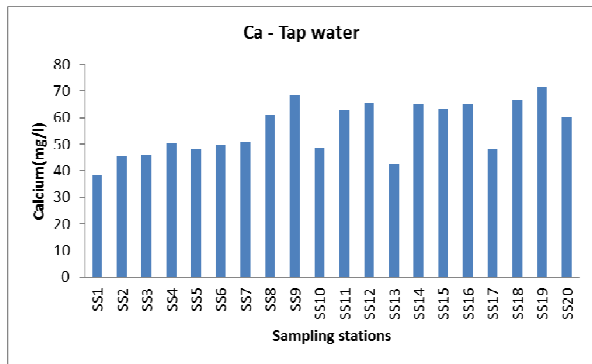
	pH	Calcium	Magnesium	Sodium
pH	1			
Calcium	0.1668	1		
Magnesium	0.0774 ^{NS}	0.149 ^{NS}	1	
Sodium	0.132 ^{NS}	0.3477	0.236	1

*Significant at 0.01level (*p <0.01), NS = Non- significant.

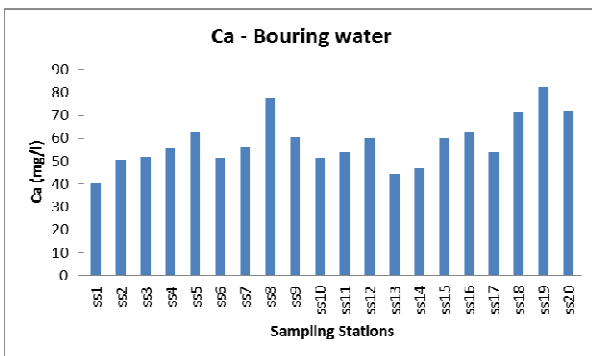
Above correlation matrix was analysed at 1% level of significance (*p <0.01).Weak correlation was observed between all the parameters of the filter water samples. This was due to ubiquitous mechanism of water filtration in different regions.

Calcium

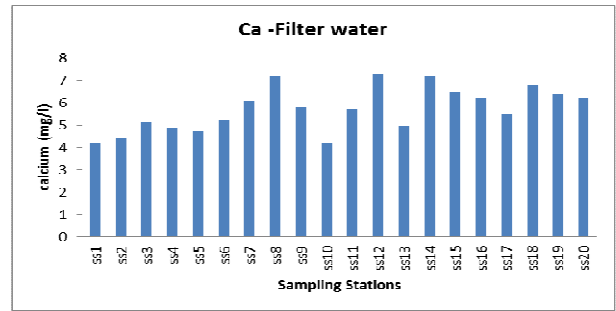
Ca levels in water sample are indicative of its hardness. Higher the Ca levels, high is its stone causing capacity. Extra intake of Calcium other than that provided by diet in the human body definitely leads to renal calculi. Average Calcium in tap water for Ghaziabad (45mg/l), Noida (52.4mg/l), Haryana (61mg/l), N & East Delhi (58.95 mg/l) and S &West Delhi (61.5mg/l) has been recorded as 45mg/l, 52.4mg/l, 61mg/l, 58.95mg/l and 61.5mg/l respectively. Amongst these highest level was recorded in SS9 (68.4mg/l) and lowest was in SS1 (38.3mg/l). Average Calcium levels in Boring water has been calculated as Ghaziabad (49.5mg/l), Noida (61.95mg/l), Haryana (56.5mg/l) and N & East Delhi (53.57mg/l) and S &West (69.925mg/l) has been recorded as 49.5mg/l, 61.95mg/l, 56.5mg/l, 53.57mg/l and 69.925mg/l respectively. This indicates highest Calcium levels were reported from Delhi south and west region in which SS19 (82.4mg/l) recorded highest Ca levels and lowest Calcium levels were detected in Ghaziabad SS1 (40.2mg/l). From the filter water analysis, drastic reduction in Ca levels has been detected. Average calcium values in filter water for Ghaziabad (4.65mg/l), Noida (5.4mg/l), Haryana(5.75mg/l), N and East Delhi(6.225mg/l) and S and West Delhi(6.225mg/l)has been recorded as 4.65mg/l, 5.4mg/l, 5.75mg/l, 6.225mg/l and 6.225mg/l respectively. However, highest Ca levels in filter water has been observed in SS12 (7.3mg/l) and lowest in SS1 (4.2mg/l) and SS10 (4.2mg/l).



Graph 1. Ca tap water of 20 sampling stations



Graph 2. Ca boring water of 20 sampling stations

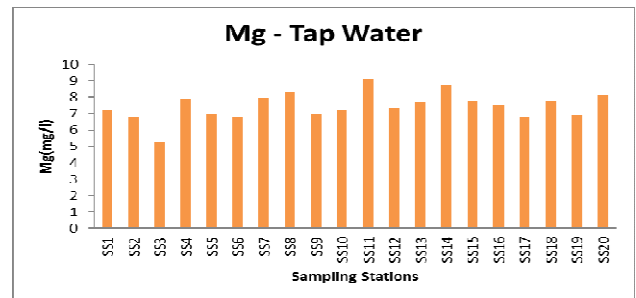


Graph 3. Ca of filter water of 20 sampling stations

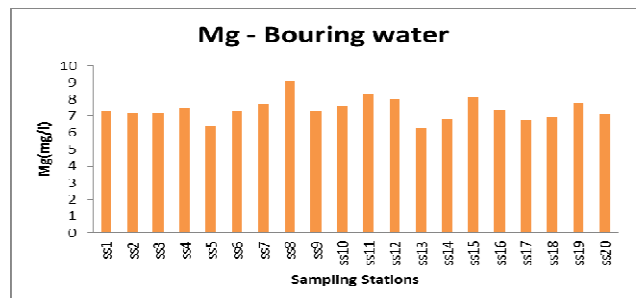
Magnesium

Magnesium levels have shown to be inversely proportional to the formation of stone .In fact magnesium supplements have been given as treatment to renal calculi patients.

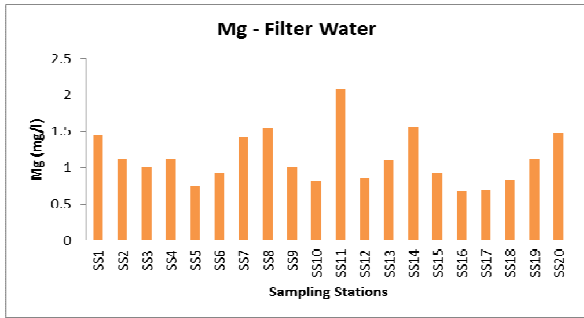
The average magnesium values in tap water has been recorded as Ghaziabad (6.8mg/l), Noida (7.5mg/l), Haryana (7.65mg/l) and N and East Delhi (7.92mg/l) and S &West Delhi (7.4mg/l) 6.8mg/l, 7.5mg/l, 7.65mg/l, 7.92mg/l and 7.4mg/l respectively. Amongst these SS1 (9.1mg/l) has highest magnesium level whereas SS2, SS6 and SS17 has lowest magnesium levels i.e.6.8mg/l The average values of magnesium for Boring water has been recorded as for Ghaziabad(7.3mg/l), Noida (7.625mg/l), Haryana(7.8mg/l), N &E. Delhi (7.15mg/l) and S & W. Delhi (7.12mg/l) as 7.3mg/l, 7.625mg/l, 7.8mg/l, 7.15mg/l and 7.12mg/l respectively. Amongst these SS8 of Noida had highest Mg levels of 9.1mg/l and lowest in N & E. Delhi SS13. The average values of Magnesium for filter water has been recorded for Ghaziabad(1.75mg/l), Noida(1.15mg/l), Haryana(1.18mg/l), N & E. Delhi(1.07mg/l), and S & W. Delhi(1.03mg/l) as 1.75mg/l, 1.15mg/l, 1.18mg/l, 1.07mg/l and 1.03mg/l respectively. Highest Mg was observed in SS11 while lowest was measured in SS16.



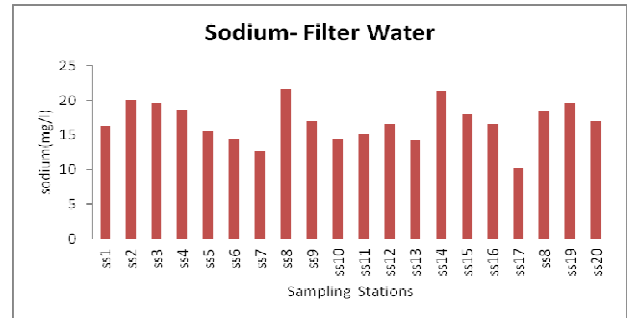
Graph 4. Mg in tap water of 20 sampling stations



Graph 5. Mg in filter water of 20 sampling stations



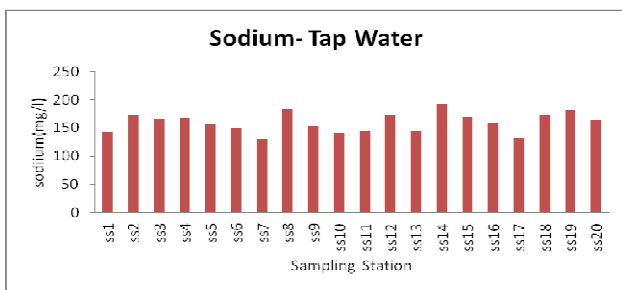
Graph 6. Mg in filter water of 20 sampling stations



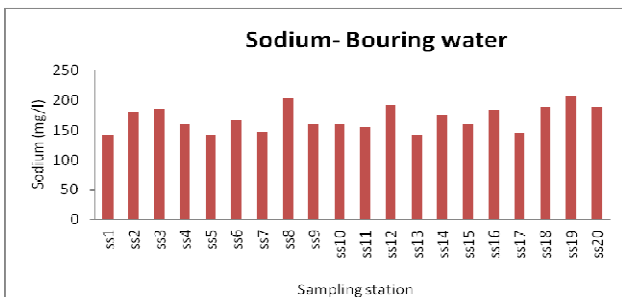
Graph 9. Sodium in filter water of 20 sampling stations

Sodium

Sodium level in water sample has recorded a high in Delhi-NCR region. The U.S. EPA advisory limit for sodium in drinking water is 20 mg/l. High intake of sodium from water is a cause for renal calculi. The obtained average values of Sodium in tap water for Ghaziabad (161.575mg/l), Noida (155.1mg/l), Haryana (152.4mg/l), N & E. Delhi (166.6mg/l) and S & W. Delhi (162.7mg/l) are 161.575mg/l, 155.1mg/l, 152.4mg/l, 166.6mg/l, and 162.7mg/l respectively. The highest sodium levels were recorded for SS14 (192.8mg/l) while lowest was SS7 (131.2mg/l). The average sodium concentration for boring water for Ghaziabad (166.5mg/l), Noida (164.65mg/l), Haryana (166.525mg/l), N & E. Delhi (165.45mg/l) and S & W. Delhi (182.12mg/l) are as 164.65mg/l, 166.525mg/l, 165.45mg/l, and 182.12mg/l respectively. Amongst these the highest sodium concentration was for SS19 (205.3mg/l) and lowest was for SS1 (140.6mg/l). Sodium levels were drastically reduced in filtered water. The average sodium values obtained for Ghaziabad (18.625mg/l), Noida (16.02mg/l), Haryana (15.825mg/l), N& E. Delhi (17.575mg/l) and S& W. Delhi (16.275mg/l) were recorded as 18.625mg/l, 16.02mg/l, 15.825mg/l, 17.575mg/l and 16.275mg/l respectively. Amongst the stations SS8 (21.5mg/l) had highest sodium whereas lowest was in SS17 (10.2mg/l).



Graph 7. Sodium in tap water of 20 sampling stations



Graph 8. Sodium in boring water of 20 sampling stations

Mg-Ca Ratio

Occurrence of renal calculi is inversely proportional to concentration of Magnesium and directly proportional to the Calcium concentration. Also magnesium-calcium ratio in water is inversely related with the incidence of the calcium-containing urinary calculus.

The Mg/Ca ratio in tap water has been very low in Delhi-NCR region which indicates that the water for intake purposes has tendency to cause renal calculi among its consumers. Particularly sample stations SS3 (0.115), SS9 (0.102), SS12 (0.111), SS16 (0.115), SS18 (0.117) and SS19 (0.096) had lowest Mg/Ca ratio which indicates their capacities to cause stone. And comparatively SS1 (0.187) and SS13 (0.181) have lower tendencies among them.

Among the Delhi-NCR boring water the ratio of Magnesium/Calcium has been particularly low which indicates their obvious and inherent stone causing capacities. Among them boring water sample from SS18 (0.096), SS19 (0.094), SS20 (0.098) and SS5 (0.101) have least Mg/Ca ratio which means increased tendency to cause stone among all. And samples from SS1 (0.181) and SS11 (0.153) have comparatively lesser stone causing capacities. With low Mg/Ca ratio the boring water in Delhi-NCR has highest stone causing capacity compared to supply tap water and filtered water.

Quite a variation has been observed in the values obtained through filtered water. High to low ratio has been recorded. This can be attributed to various makes and design and variation among the equipment and their usage. However of the obtained values SS12 (0.116), SS15 (0.143), SS16 (0.109), SS17 (0.127), SS18 (0.122) still retained capacity to cause stone. Filtered water from sampling station SS1 (0.345) and SS11 (0.364) and SS2 (0.256) had decreased capability to cause stone.

CONCLUSION

Kidney stones often have no definite, single cause, although several factors may increase your risk. Kidney stones form when your urine contains more crystal-forming substances — such as calcium, oxalate and uric acid — than the fluid in your urine can dilute. At the same time, urine may lack substances that keep crystals from sticking together, creating an ideal environment for kidney stones to form.

Besides geographical consideration like climate in Delhi-NCR region and genetic factors from the lithogenic as well as aquagenic analysis following conclusions can be drawn.

The major reason for high incidence of stone is excessively high intake of sodium through both supply and boring water as well as food items. Moreover with the coming of canned food high in

sodium this has been the major cause for formation of renal calculi. High sodium results in excessive excretion of calcium which leads to development of stones.

Also Indians prefer lot of vegetables. Most of the oxalate containing vegetable is healthy, with the result, people tend to overtake these vegetable like tomato, beans, spinach, carrot. The vegetables are always complementary along with pulses. So both the factors i.e. Oxalate and protein come together to obviously develop stones.

Also the Indian tea and coffee which is frequently consumed leads to concentrated urine providing super-saturation of crystals. While drinks like alcohol and cold-drinks too cause stone.

Though from the population surveyed it has been cleared that Indians do not prefer Ca, vitamin D supplements. However, the excess of Calcium is going through the water intake. The Ca obtained through diet surely does not harm and effect renal calculi formation. But this over Ca from water gets concentrated and making people susceptible to calculi formation.

Also it has been found that Indian diet lacks potassium, magnesium and citrate elements in adequate amounts. Their intake leads to inhibition of calculi formation. However Magnesium supplied through water is cancelled by the extra doses of calcium supplied.

The diet lacks fruits, fibres and lot of fluids. Numbers of people having cold-drink were much higher than the people consuming juice. So a healthy and active lifestyle must be incorporated.

It is strongly recommended that eat everything in adequate yet limited quantity hygienically and consumption of soft water is highly required to prevent and avoid recurrence of kidney stones.

REFERENCES

- [1] Wedro B., A report on kidney stones, http://www.emedicinehealth.com/kidney_stones/article_em.htm
- [2] <http://timesofindia.indiatimes.com/city/ludhiana/Kidney-stone-cases-rising-in-kids/articleshow/12943830.cms>
- [3] <http://www.nejm.org/doi/pdf/10.1056/NEJM199303253281203>
- [4] Paterson, R; Fernandez, A; Razvi, H; Sutton, R 2010. "Evaluation and medical management of the kidney stone patient". *Canadian Urological Association Journal* 4 (6): 375–9. PMC 2997825. PMID 21191493.
- [5] Taylor, EN; Curhan, GC 2006. "Diet and fluid prescription in stone disease." *Kidney international* 70 (5): 835–9. doi:10.1038/sj.ki.5001656. PMID 16837923.
- [6] http://www.nmcth.edu/images/gallery/Editorial/y8fPQSanjiv_Risal.pdf
- [7] www.ictm.tn.gov.in/dengue/Renal20%/description1.htm
- [8] Johnson, Dana 1998. "RemovingBeerstone". *Modern Brewery Age*. Birko Corporation R&D. Retrieved 2007-08-06.
- [9] http://en.wikipedia.org/wiki/Uric_acid
- [10] <http://www.ncbi.nlm.nih.gov/pubmed/17148700>
- [11] <http://www.ncbi.nlm.nih.gov/pubmed/16698380>
- [12] <http://causesof-kidneystones.blogspot.in/>
- [13] <http://www.icm.tn.gov.in/dengue/Renal%20stone/description1.htm>
- [14] <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3126068/>
- [15] <http://www.europanurology.com/article/S1569-9056>
- [16] http://www.medicinenet.com/kidney_stone/page2.htm
- [17] <http://semj.sums.ac.ir/vol2/apr2001/nephrolithiasis.htm>
- [18] http://doctor.ndtv.com/faq/ndtv/fid/8500/Does_drinking_hard_water_lead_to_kidney_stones.html.
- [19] Impact of Nutritional Factors on Incident Kidney Stone Formation, the *Journal of Urology*.
- [20] Nutrition Research, Urolithiasis – Nutritional Aspects, associated the increase in the incidence of kidney stones with “affluence”, 1983.
- [21] <http://holistichealthinsider.com/kidney-stones-what-simple-things-increase-or-decrease-your-risk>.
- [22] http://doctor.ndtv.com/faq/ndtv/fid/8500/Does_drinking_hard_water_lead_to_kidney_stones.html.
- [23] APHA-AWWA, 2005. Standard method for the examination of water and wastewater ,21st ed. Washington, D.C.