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Research Article

Correlation of biochemical constituents present in kidney stones and diet from an Indian population

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ABSTRACT

Renal calculi (or kidney stones) post surgical intervention from the various hospitals in the Mumbai region were analyzed for the presence biochemical components such as calcium, magnesium, oxalate, phosphate, sulphonamide, cholesterol and carbonate at our department. In this study, we retrospectively analyzed 250 kidney stone samples which were characterized biochemically during the period 2008-2014 to evaluate the predominant component and to establish any correlation which may exist between the biochemical components in these samples and the diet of these patients. The population was statistically analyzed using the Z-proportion test for age, gender and diet; diet was segregated into a moderately non-vegetarian (MNV) and non-vegetarian (NV) and presence of the each of the above components. Majority of the samples presented to our centre were from male subjects. Children were more susceptible to the presence of sulphonaminde and carbonate stones. Women more prone to phosphate stones and moderate non-vegetarians were more prone to calcite stones. Taken together, the data suggest that all three factors age, gender and diet can influence composition of stones.

Key words: *Composition of renal calculi, age, gender, diet.*

INTRODUCTION

Renal calculi (or kidney stones) have affected mankind since history began and the disease appears in many parts of the world with variable intensity. High incidence areas are Scandinavian countries, Mediterranian-British isles, Central Europe, Northern Australia, parts of Malaysia, China, Pakistan and North-western India[1]. The origin of many stones is related to the amount of their constituents excreted into the urine. When solids precipitate out of a solution, it is generally because the concentration of the solute exceeds it solubility. Solutions where the concentration of solute is in between the concentration of precipitation and the solubility are metastable. The urine is normally supersaturated with respect to calcium oxalate and therefore most urines are metastable, whether they are from normal or stone-forming individuals. Normal urine contains citrate that inhibits formation of calcium-containing crystals. Proteins such as calgranulin (an S-100 calcium binding protein), Tamm-Horsfall protein, glycosaminoglycans, uropontin, nephrocalcin, prothrombin F1 peptide, and bikunin present in urine also act as inhibitors to stone formation. However, when these substances fall below their normal proportions, the urine becomes supersaturated with one or more solutes which can precipitate out, a seed crystal may be formed by the process of nucleation. Other solutes within urine deposit onto the seed crystal which serves as the solid scaffold and heterogenous nucleation proceeds. This process speeds up when urinary pH is unusually low or high. Thus calculi can be segregated into three categories: simple calculi, which contain a single urinary constituent; mixed calculi, which contain two or more constituents and foreign body calculi, which may be present due to introduction of some constituents from outside the body and their size can vary considerably from little more than a pinch to the size of an egg. Generally the largest stones are found in the bladder whilst stones from kidney and the renal pelvis are smaller and in some cases small enough to pass long the urethras and to be passed in urine. But very large stones can be found in kidneys particularly in hydro-nephrosis. Majority of the urinary stones have been biochemically characterized by various authors and have been predominantly described as calcium oxalate crystals. Dietary factors are believed to play an important role in formation of kidney stone and diets containing high amounts of oxalate, animal protein, sodium, high fructose increases the chance of stone formation whereas increase in fluid consumption, magnesium, potassium and vegetables which help in maintaining the pH at neutral value all help to inhibit stone formation[2]. Quality of water ingested and ambient temperature also seem to play a role in stone formation. Underlying metabolic diseases such as Crohn's disease, distal renal tubular acidosis, Dent's disease, and hyperparathyroidism also contribute to the formation of stones. Data on the influence of diet on stone formation have been derived by experiments individuals put on a varying diets rich in one or more components and a biochemical characterization of urine secreted. Structural analysis of kidney stones through IR spectroscopy, raman spectroscopy, scanning electron microscopy and X-ray crystallography[3] etc. have also been carried out. Propensity for stone formation has been investigated by controlling the diet of healthy volunteers[4,5,6]. Biochemical characterization of stones and their differentiation based on biochemical component present have also been carried out by various authors[7]. However, to our knowledge, there have been no studies that correlate diet of the subject to the actual biochemical composition of the stones retrieved post surgery. In this study, we examine the correlation between diet and biochemical composition of mixed stones retrieved from patients who underwent surgery in hospitals in and around the Mumbai region of Western India.

MATERIALS AND METHODS

The study included 250 kidney stones samples of patients which came from various hospitals in the Mumbai region post-surgery. Samples were post-surgically delivered to our center with subjects consent. All patients included were those who had surgery for renal calculi for the first time. Subjects were primarily divided by age, gender and diet. Subjects aged < 18 yrs were considered as children. Subjects were classified as mild non-vegetarians (MNV) when their diet was completely devoid of meat or included consumption of white meat (chicken / fish) once a week. Subjects were classified as non-vegeterians (NV) when their diet included both white and red meat (beef / mutton / chicken / fish) and was consumed atleast 5-6 days a week.

Inclusion criteria

Most of the samples received at our center were mixed samples, however some of the samples analysed were positive for 2 components only. In this study we selected samples which were positive for 4 or more components.

Initial processing of the stones

Stones were washed with tap water to remove debris, dried completely by incubation at 60ºC and weighed. The stone sample crushed in a mortar & pestle to make a fine powder prior to analysis. All chemicals required for biochemical characterization of stone samples were AR grade and were procured from Sisco Research Laboratories (SRL, Mumbai).

Biochemical Analyses of various components

All analyses were carried out as per the procedures described in Hodgkinson (1971)[8]. In brief, presence of calcium was detected by observing the formation of white precipitate of calcium oxalate at high temperatures in the presence of dil. HCL and ammonium oxalate using conc. ammonia and acetic acid to adjust the pH during the reaction. Magnesium was detected by filtering the above solution of calcium oxalate through a filter and reacting it with titan yellow at alkaline pH (excess KOH) and formation of a red colored flocculent precipitate of magnesium hydroxide. Presence of oxalate was detected by observing the decoloration of potassium permanganate solution in the presence of sulphuric acid at 60-70 ºC. Phosphates were detected by formation of a canary yellow precipitate of ammonium phosphomolybdate on reacting the sample with ammonium molybdate in the presence of concentrated nitric acid.Suphonamides were detected using the majenta color formation in presence of sodium nitrite in presence of ammonium sulphamate and sulpha dye at acidic pH. Carbonate was detected following release of CO_2 from the kidney stones upon treatment with HCl.

Statistical Methods

Calculation of presence of the biochemical components:

The presence of a biochemical component was calculated as: Percentage of samples positive for a component
= (No. of samples positive for a component / total number of samples)*100

***Two proportion z-test*:**

Since the population size was 250, and the sub-categories were qualitative, the 2-proportion z-test was applied to determine the significance of the presence of different biochemical components in the kidney stones

The Z score was calculated using the formula

$$Z = p1 - p2 \; / \; \{\sqrt{[p(1-p)\,(1/n1 + 1/n2)]}\}$$

Where

p = total proportion = number of samples positive for uric acid / total number of samples (e.g. no. of males + no. of females)

p1 = proportion of first population (e.g. no. of uric acid positive samples in females / total number of females)

p2 = proportion of second population (e.g. no. of uric acid positive samples in males / total number of males)

n1 = number of samples in first population (e.g. no. of females)

n2 = number of samples in second population. (e.g. no. of males)

The absolute value of the z-score was then used to read the P (probability) for the two tailed test.

RESULTS AND DISCUSSION

About 90% of the samples presented at our center were positive for calcium and oxalate similar to all previous data reported earlier[3]. Approximately 90% of the samples were positive for calcium oxalate. About 40% were positive for magnesium and the presence of magnesium was not mutually exclusive to the presence of calcium in the same sample. Phosphate was also present in 40% of samples whereas 10% of samples were positive for carbonate, and 5% were positive for cholesterol and sulphonamide (Figure 1). Out of the 250 samples analyzed in this study, 30% were that from children. Figure 1 shows the comparison of the prevalence of biochemical component occurrence among adults and children. When the prevalence was separated on the basis of age, the prevalence of the various components in adults followed the general trend, but samples from children had more prevalence of phosphate, sulphonamide and cholesterol compared to adults. In contrast, adults had a greater prevalence of carbonate compared to those from children. To verify whether these differences were statistically significant, we used the 2 tailed Z test (Table 1).

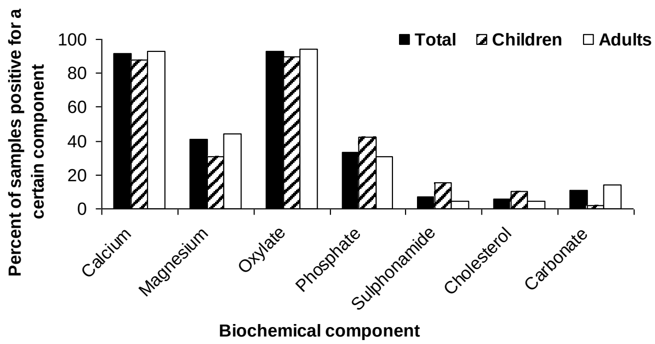

Figure 1: Analyses of 250 kidney stones on age of subjects. Total = adults + children; Y axis depicts the percentage of samples which were positive for a particular component.

Table 1: Two tailed Z-test analysis for phosphate, sulphonamide, cholesterol and carbonate in all samples based on age

	T-C	T-A	T-C v/s T-A	T-C	T-A	T-C v/s T-A	T-C	T-A	T-C v/s T-A	T-C	T-A	T-C v/s T-A
	Phosphate			Sulphonamide			Cholesterol			Carbonate		
No. positive	25	59		9	8		6	8		1	27	
N	59	191		59	191		59	191		59	191	
Proportion	0.424	0.309		0.153	0.042		0.102	0.042		0.017	0.141	
Z value			**1.64**			**2.96**			**1.75**			**-2.64**
p value			**0.103**			**0.003**			**0.08**			**0.008**

Where T-A = Total adults, T-C = total children. $p < 0.05$ is considered to be significant.

Table 2: Two tailed Z-test analysis for phosphate, sulphonamide, cholesterol and carbonate in all samples based on gender

	M	F	M v/s F	M	F	M v/s F	M	F	M v/s F	M	F	M v/s F
	Phosphate			Sulphonamide			Cholesterol			Carbonate		
No. positive	60	24		13	4		9	5		19	9	
N	198	52		198	52		198	52		198	52	
Proportion	0.303	0.462		0.066	0.077		0.045	0.096		0.096	0.173	
Z value			**-2.16**			**-0.28**			**-1.4**			**-1.57**
p value			**0.0308**			**0.78**			**0.15**			**0.116**

Where M = Total males, F = Total females. $p < 0.05$ is considered to be significant.

Table 3: Two tailed Z-test analysis for calcium, magnesium and phosphate in all samples based on diet

	M-NV	NV	MNV v/s NV	M-NV	NV	MNV v/s NV	M-NV	NV	MNV v/s NV
	Calcium			Magnesium			Phosphate		
No. positive	143	86		71	32		51	33	
N	158	92		158	92		158	92	
Proportion	0.905	0.544		0.449	0.203		0.323	0.209	
Z value			**6.56**			**3.92**			**1.94**
p value			**0.000**			**0.001**			**0.053**

Where MNV = Moderate non-vegetarians NV = non-vegetarians. $p < 0.05$ is considered to be significant

While the difference in phosphate and cholesterol are insignificant ($p = 0.103$ and 0.08, respectively i.e. $p > 0.05$), the differences in sulphonamide and carbonate are significant ($p = 0.003$ and 0.008, respectively i.e. $p < 0.05$). However, the nature of the z values tell us that there is a higher probability of the presence of sulphonamide in stones from children than adults, whereas there is a higher probability of carbonate in the stones of adults compared to children. We next analyzed the data based on gender (Figure 2).

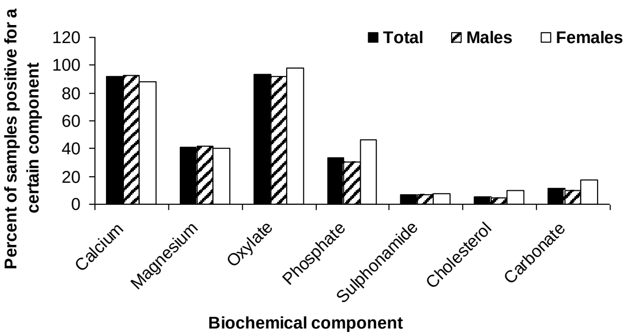

Figure 2: Distribution of components of renal calculi based on gender. Y axis depicts the percentage of samples which were positive for a particular component.

While there were no significant differences in most of the components, a higher proportion of samples from female subjects seemed to have phosphate and carbonate in them. We analyzed this higher proportion by the z-test and the results are presented in Table 2.

No. of samples containing phosphate in females was significantly higher than that in males ($p = 0.04$). Finally we analyzed the data based on diet (Figure 3).

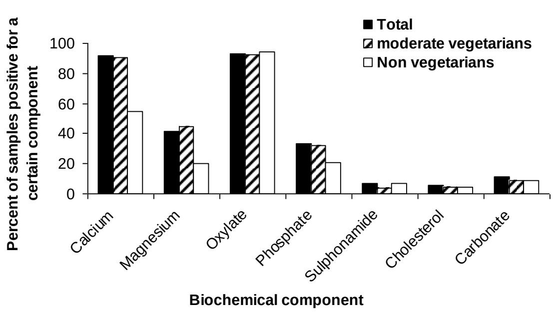

Figure 3: Distribution of components of renal calculi based on diet. Y axis depicts the percentage of samples which were positive for the various components.

A z-test analysis of the components reveals that calcium, magnesium & phosphate are predominantly present in moderate non vegetarians compared to vegetarians (Table 3)

DISCUSSION

Majority of the renal calculi referred to our center were from males suggesting that they were more prone to calculi formation. Furthermore >95% of the calculi were mixed stones containing a variety of biochemical components. Of all samples, 90% of the samples were predominantly positive for both calcium and oxalate suggesting that calcium oxalate stones were the predominant type of calculi present in the population studied herein. Sulphonamides in diet are largely from the brassicas or cruciferous vegetables such as broccoli, cauliflower, cabbage and brussels sprouts and the allium vegetables, which include onions, garlic, leeks, shallots and chives. Sulphonamides have been commonly used treat microbial infections and urinary tract infections in children and often have a side effect of causing kidney stones[9,10]. Urinary tract infections are the most common pathologies observed in children atleast as observed in 3 centers[11,12,13]. We also observed a significant increase in the number of carbonate stones from adults compared to children. This corroborates well with the observation that carbonate apatite stones are more prevalent in adults compared to children[14]. Our analysis based on gender showed that stones from females have higher phosphate content than males, this corresponds well with the study by Daudon *et al.*, which looked at 27,980 calculi, collected between the years 1976 and 2001, analyzing their composition via infrared spectroscopy, found that females tend to have a preponderance for calcium phosphate and struvite stones, presumably due to increased susceptibility to urinary infections[15]. Almost all investigators from the early seventies have implicated dietary factors in the causation of stone[16]. Only eight foods--spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries cause a significant increase in urinary oxalate excretion[17]. Out of these spinach, nuts, tea and wheat bran are important ingredients of the Indian diet. In particular, studies in Indian children have shown that consumption of whole wheat flour as a staple food leads to the production of urine with a high specific gravity (SG), increased excretions of calcium, magnesium and phosphorus[18]. Our data corroborates with this observation and male moderate non-vegetarians having a high carbohydrate diet had a significantly greater probability of having calcium and magnesium in their stones (Table 3). There is emerging evidence that renal calculi could be an indicator of the metabolic syndrome and/or type 2 diabetes in humans[19.20]. Given the rising incidence of type 2 diabetes in India, the correlation of diet, lifestyle and renal calculi composition could provide insights to the metabolic pathways impaired during development of the metabolic syndrome and its influence on renal function.

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