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Chemical Composition and Epidemiological Risk Factors of Urolithiasis in Ardabil Iran

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Abstract: The aim of this study was to perform the chemical analysis of stones to investigate the pattern of biochemical composition of stones and to determine epidemiological risk factors for stone formation in Ardabil province of Iran. Renal stones from 1268 patients were analyzed during 5 year period from March 2001-March 2006. These stones were sent to seven laboratories in 2 cities of Ardabil province for analysis from different clinics and hospitals. The stones were analyzed by semi-quantitative method (Saba kit). The powdered stones and standards both were analyzed for uric acid, cystine, oxalate, phosphate, ammonium, calcium and magnesium contents. Male to female ratio was 2.7:1. The stone frequency was very greater in adults as compared to children. Maximum number of stones was analyzed in summer months. Calcium oxalate stones were the commonest followed by uric acid and phosphate stones. Only 8 cystine stone was found in the series analyzed. From the study of epidemiological factors, it seems that the men are more prone to development of stones. A clear stone season seems to exist in the area corresponding to the summer months. The relative increased frequency of stones in the region indicates that nutritional, environmental and genetic factors play a role in the occurrence of stones. M/F ratio, male to female ratio; mm, millimeters; mg, milligrams

Key words: Urinary stones, composition, chemical semi-quantitative analysis, epidemiology, M/F ratio, stone wave

INTRODUCTION

Urinary calculi are for the human race and the more so for male individuals, one of the most intolerable diseases that life can be afflicted with. They are found in about 1% of all autopsies (Vinay *et al*., 2007). Urolithiasis or formation of urinary calculi at any level of the urinary tract is a common condition, but most often the calculi arise in the kidney.

Urinary calculi are worldwide in its distribution but the overall probability of forming stones varies in different parts of the world. The risk of developing nephrolithiasis in normal adults appears to be lower in Asia (1-5%) than Europe (5-9%) and North America (12% in Canada, 13% in USA). The highest risk was reported in Saudi Arabia (20.1%) (Adriano *et al*., 2000). Prevalence rate is 4-15% in males and 4-8% in females and incidence rate varies from area to area: 53.2 per 100,000 population in Japan, 364 in Malaysia and 540 in West Germany. Prevalence and/or incidence rates have, in general, increased in the developed countries since World War II and in the developing countries as well, where upward trends are quite analogous to the trends observed in the 19th century in Europe (Kodama *et al*., 1989). The incidence of urinary tract stones in Japan has also increased steadily over the past 30 years and seems that will continue to do so in the near future (Yoshida *et al*., 1999).

The M/F ratio (Male to Female ratio) is about 2-4. The peak incidence is observed in 2nd and 3rd decades of life and most of present patients are in 5th decade of life. Renal calculi are characterized clinically by typical intense pain known as renal or ureteral colic as they pass down along the ureter and manifest as gross hematuria. The stones have capacity to obstruct urine flow or to produce trauma and ulceration. Sometimes urinary stones predispose to urinary infections (Vinay *et al*., 2007).

There are different types of calculi. Most stones about 75-80% are calcium containing composed largely of calcium oxalate followed by calcium phosphate. Another 10-15% are struvite stones composed of magnesium

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ammonium phosphate. Six percent are uric acid stone and 1-2% are made up of cystine stones. An organic matrix of mucoprotein makes up about 2.5% of all stones by weight (Scheriner *et al*., 1991; Vinay *et al*., 2007).

The epidemiology of urolithiasis differs according to geographical area and historical period: changing socio-economic conditions have generated changes in the incidence and type of lithiasis in terms of both the site and the physical-chemical composition of the calculi. Reno-ureteral calculosis typical of adult age and featuring mainly calcium oxalate and phosphate is currently more frequent in economically developed countries. On the contrary, primitive vesical calculosis is fairly widespread in developing countries, with calculi composed of ammonium urate and calcium oxalate. Vesical calculosis can arise due to malnutrition in the very early years of life and its incidence is decreasing in proportion as social conditions gradually improve. In the course of the last 100 years, there has been a gradual decrease in incidence of vesical calculosis in Europe, Northern America, Australia and Japan, while the reno-ureteral calculosis has become more common. This trend defined as stone wave has been explained in terms of changing social conditions and the consequent changes in eating habits (Trinchieri, 1996).

Renal stones formation is believed to be the result of both excessive concentration of the stone constituents and some physicochemical situations. Important factors are excessive concentration of urinary excretory products because of highly concentrated urine, resulting from environmental or habitual chronic dehydration. Hypercalciuria from various causes, excessive oxalate or uric acid production or an acquired or genetic basis and hereditary cystinuria are important causes. The factors conducive to precipitation of crystalloid may be equally important. An alkaline pH favors calcium phosphate stone formation (Jager, 1996).

So, these metabolic derangements are found in patients with idiopathic calcium stone disease: idiopathic hypercalciuria in 49.5% of patients, hyperoxaluria in 27.8%, hyperuricosuria in 23.0%, hypocitraturia in 22.6 and low urine volume in 10.9%. 18.8% of patient has not any identifiable metabolic changes (Adriano *et al*., 2000).

Analysis of 24 h urine specimens from stone-forming patients reveals seasonal variations in urinary risk factors for nephrolithiasis. Urine volume, pH and sodium are significantly lower during the summer than in the winter. These changes are consistent with summertime sweating and increased physical activity. The decrease in urine pH cause relative supersaturation of uric acid, which is far more marked in men. The summertime sodium depletion is modest and urine calcium is not increased in this time, but in male patients, remarkable decrease in urine volume cause high calcium oxalate supersaturation. Females have maximum calcium oxalate supersaturation in early winter because of decreasing urine volume and increasing urine calcium excretion (Stuart *et al*., 1991; Parks *et al*., 2003).

Since the chemical composition of urinary calculi is very important for the purpose of determining both the origin and etiology, a study was therefore, done to perform the chemical analysis of stones to know the pattern of biochemical composition of stones in Ardabil province of Iran.

MATERIALS AND METHODS

The stone were sent to seven laboratories in 2 cities of Ardabil province of Iran (Ardabil and Khalkhal), for chemical analysis, from different clinics and hospitals, during 5 year period from March 2001-2006; this time period is equal to 5 complete Iranian solar years, from 1380-1384. These laboratories were the only centers which were performed urinary stone analysis in Ardabil province. The stones were passed spontaneously in nearly half of the patients and were removed surgically in others. These stones were washed in distilled water, grinded and powdered in a mortar and were analyzed by semi-quantitative method.

The kits supplied by Saba laboratory (Iran) was used for chemical analysis of stones. The synthetic control available in the form of fine power was analyzed along with the test samples. The powered stones and control both were analyzed for uric acid, cystine, oxalate, phosphate; ammonium, calcium and magnesium contents. Finally, the data was analyzed by Excel software program.

RESULTS

Total 1268 stones were analyzed chemically during 5 year period from March 2001-2006. Both adults and children were included in the study.

Of 1268 urolithiasis patients that we reviewed in total, 919 patients were male, while 346 patients were female. The M/F ratio was 2.7:1 (Table 1). This ratio was different for stones having some compositions; notably 5.5 for ammonium magnesium phosphate (struvite) 1.7 and 1.6 for stones containing cystine and calcium phosphate, respectively. Ammonium urate containing stones are noted only in male patients. Similar relations were also found in patients with pure stones; M/F ratio was 1.7 and 1.3 in pure stones formed of cystine and calcium phosphate, respectively. Interestingly, all of 5 pure struvite stones were found in male patients; The M/F in the latter group was significantly different from total patients (p value < 0.0001). The M/F ratio was also larger

in lower age groups; 4.3 in patients below 20 years old and 2.0 in patients above 50 years old. So, sexual difference was greater in ages under 20.

The patients were ranging in age from 2 -86 years. They had a mean age of 40.5 ± 15.1 years (mean ± SD). Mean age was different in females and males, 42.2± 15.5 and 39.8± 14.9 years, respectively. The mean ages of patients having stones predominantly formed of uric acid were 49.7 ± 15.9, 53 ± 14.2 and 48.2 ± 16.4 years (mean ± SD), for total patients, female and male subjects, respectively. The ages of these patients were not differ significantly from each other (p=0.054), instead uric acid stone formers were significantly older than total patients, which were also true in both sex groups (p values < 0.0001).

The range of dimensions of the stones was 2-60 mm; 78.4% of them were smaller than 10 mm and only 2% of them were typical staghorn, which were greater than 4 cm in length.

The weights of stones were ranging from 5-97000 mg (mean, 1011). Weight of 75% of stones was under 500 mg, 2.1% were heavier than 10,000 mg (staghorn) and only two weigh more than 50,000 mg. Although there was no trend between sex and the weight of stones (mean, 1166 mg for men and 950 mg for women), the weight of stones in patients younger than 40 years old were insignificantly lower than ages above 40 years; mean, 595 and 1185 mg, respectively (p value, 0.062). The weights of stones shows considerable variation in different times of year; winter and autumn had a greater number of heavy stones than summer and spring; average weights were 1230, 1099, 941 and 808 mg, respectively. These differences were not significant too (p value, 0.15). In February and April, significantly lighter stones were analyzed, mean weights 389 and 444 mg, respectively (p values <0.0001, 0.0008). The heaviest stones are seen in January with a mean weight of 2030 mg (p value, 0.133). Chemical composition of stones appears to have remarkable effects on weight of stones, namely struvite stones were significantly heavier and cystine stones were lighter than others; their mean weights were 5903 and 553 mg, respectively (p values, 0.018, 0.015). On the other hands, struvite constitutes more proportion of heavier stones, i.e. 3.4%, 6 and 10% of total stones, stones heavier than 4000 and than 7000 mg, respectively (Table 2).

Out of a total of 1268 stones, pure stones were seen in 679 patients (53.4%) and mixed stones in 591 patients (46.6%). A stone was defined as pure, when more than 95% of its weight formed of a specific composition. Five hundred and forty five pure stones (80.3% of them) were formed of calcium oxalate; uric acid, cystine and calcium phosphate are the next ones and their frequencies are 18.6%, 0.7 and 0.4% (Table 3).

Calcium oxalate was the most predominant composition in stones of 1017 patients (80.2% of total). Uric acid, calcium phosphate, cystine and struvite were predominant compositions in 205 (16.2%), 30 (2.4%), 8 (0.6%) and 5 (0.4%) patients, respectively (Table 4). On the other hand, mere presence of a chemical composition was found in a different numbers of stones; calcium oxalate, uric acid, calcium phosphate, struvite, ammonium urate and cystine are present in 1122, 366, 281, 43, 23 and 8 patients, respectively (Table 5).

Ammonium urate was found only in 23 stones (1.8% of total); its average amount in these 23 stones was 19.5%. Ammonium urate in combination with calcium oxalate formed 87% of stones in these 23 patients. Of these, only 7 patients (30%) were less than 20 years old. Only one stone was predominantly formed of ammonium urate (Table 5). In patients having ages less than 20 years old, calcium oxalate was the most common predominant composition (81%) and uric acid (13%), calcium phosphate (3%) and cystine (3%) were subsequent ones. No patient had stones predominantly formed of ammonium urate.

Maximum number of stones was analyzed in summer season (Table 6 and 7). Winter, spring and autumn were subsequent seasons. In men, frequency of calcium oxalate and uric acid stones were greater in summer months, 219 and 51 subjects, which were 17.6 and 41.7% greater than average amounts (p values, 0.0042 and 0.0045). Accordingly, the lowest numbers of predominantly uric acid stones were present in autumn, in 21 male patients, which was 41.7% lower than expected (p value, 0.0001). But in women, stones predominantly formed of calcium oxalate and uric acid stones had greater frequency in autumn and winter respectively; 23 uric acid stones were analyzed in winter and 80 calcium oxalate stones were analyzed in autumn; these were 50.8 and 19% greater than average amounts (Table 8). The difference was significant only for female uric acid stones in winter (p value, 0.0204).

Table 1: Sex distribution of patients

Sex	No. of patients	(%)	Ratio
Male	919	72.5	2.7 : 1
Female	346	27.3	
Unknown	3	0.2	-

Table 2: Presence of struvite in composition of stones with different weights

	Presence of struvite	Struvite>20(%)
Any weight	3.4%	1
Stones >4000 mg	6%	4.5
Stones >5000 mg	7.8%	5.9
Stones >6000 mg	9.1%	6.8
Stones >7000 mg	10%	7.5

Table 3: Chemical compositions of pure urinary stones

	Calcium oxalate	Uric acid	Cystine	Calciumphosphate
No. of patients	545	126	5	3
Percentage	80.3	18.6	0.7	0.4

Table 4: Predominant chemical compositions in all urinary stones (pure or mixed)

Stone type	No. of stones	(%)
Calcium oxalate	1017	80.2
Uric acid	205	16.2
Calcium phosphate	30	2.4
Struvite	5	0.4
Ammonium urate	1	0.1
Cystine	8	0.6
Others	2	0.2
Total	1268	100

Table 5: Chemical compositions found in all urinary stones (pure or mixed)

Stone type	No. of stones	(%)
Calcium oxalate	1122	88.5
Uric acid	366	28.9
Calcium phosphate	281	22.2
Struvite	43	3.4
Ammonium urate	23	1.8
Cystine	8	0.6
Others	2	0.2

Table 6: Month wise distribution of stones

Month	Number	(%)
January	110	8.7
February	89	7
March	65	5.1
April	114	9
May	117	9.2
June	123	9.7
July	118	9.3
August	128	10.1
September	90	7.1
October	102	8
November	91	7.2
December	121	9.5
Total	307	100

Table 7: Season wise distribution of stones

Season	Number	(%)
Winter	320	25.2
Spring	296	23.3
Summer	369	29.1
Autumn	283	22.3
Total	1268	100

Table 8: Season wise distribution of different stone compositions in two sexes

Seasons	Calcium oxalate in women	Uric acid in women	Uric acid in men	Calcium oxalate in men
Winter	61	23	39	181
Spring	58	12	33	180
Summer	70	15	51	219
Autumn	80	11	21	165
Total No.	269	61	144	745

In patients less than 20 years old, the seasons of presentation were winter in 41% of cases, summer in 31%, spring in 16 and autumn in 12%; so a significantly lower numbers of stones were noted in autumn (p value, 0.0172). Numbers of female patients under 20 were larger in winter season by 92% (p value, 0.0154). Mean numbers of patients in Ramadan month was 92, which was even lower than average numbers in similar months of cold half of the year. Ramadan is one month of the lunar year which is the time of daytime fasting for Moslems; this month was within months of autumn in time period of our study.

DISCUSSION

The present study shows that incidence of stone formation in northern west region of Iran (Ardabil) is rather high.

The mean age of the patients is 40.5 years in our study, which show a subtle insignificant sexual difference, 42.2 years and 39.8 years in males and female, respectively. Joual *et al.* (1997) reported mean age of 45 years in his patients. In Japan, Kawamura *et al.* (1991) have reported different age distribution for both sexes, average age of involved males was in the forties, while that in female was in the fifties. The average age was 46.4 years old for all patients of this study. The age of our patients is slightly lower than most studies, but slight sexual difference in age distribution is seen similar to other reports. The average age of male subjects with uric acid stones was 49 years in report of Ito *et al.* (1995) from Japan, which is agreed with our result in this subgroup (47.7 years), but the females of that study are very younger than our patients (34 versus 53 years) (Ito *et al.*, 1995).

The M/F ratio in our study was 2.7:1. Most workers have reported that this ratio is about 2 times more in male than females. Peters have reported that, it is 2-4 times more in males as compared to females. This ratio was 2.9:1 in a report from Taiwan (Lin *et al.*, 1994). Other large study was done on 1816 patients from Okinawa (Japan) and revealed M/F ratio of 2.7:1 (Rayhan Zubair Hossain *et al.*, 2003). These findings are in close proximity to our reports. We found different M/F ratios for various stone types; the highest number for this ratio is seen in patients which having struvite containing stones (5.5:1). A report from Australia show differences between M/F ratios of different stone types; which is 2.8, 3.7, 1.4 and 0.7 for Calcium oxalate, uric acid, calcium phosphate and infection stones respectively (Baker *et al.*, 1993). Our ratio for calcium oxalate is similar with this study, but M/F ratios are significantly different in other stone types. However, Australian researchers also find an increased frequency of calcium oxalate stones in women 20 -25 years of age with M/F ratio of 0.7, which is not compatible with our results. This number is 2.2 for our uric acid patients, but Ito *et al.* (1995) reported a very high M/F ratio (11:1) in patients with uric acid stones. Similarly, the M/F ratio

Table 9: Comparison of predominant chemical compositions in different studies (%)

Studies	Our study	(Adriano *et al*., 2000)	(Rayhan *et al*., 2003)	(Baker *et al*., 1993)	(Khan *et al*., 2004)	(Yoshida *et al*., 1990)
No. of cases	1.268	7.100	1.816	3.634	307	69.949
Calcium oxalate	80.2	70.1	81.6	68	78	79.4
Calcium phosphate	2.4	7.6	5.1	3	0	
Uric acid	16.2	15.2	9.6	17	19	5.2
Struvite	0.4	2.8	3.7	12	3	7.4
Cystine	0.6	1.5	0	0	0	1.0
others	0.2	2.8	0	0	0	0

was larger in lower age groups, which is not consistent with other studies; Cachat *et al.* (2004) show that M/F ratio is 2.1 in children which is comparable with adults.

Typical large staghorn stone is seen in 2% of our patients in respect of size and weight, but Joual found such stones in 12.2% of cases (Joual *et al*., 1997). Struvite stones constitute more proportions of these, which are seen also in other studies.

There are different types of calculi. Our results are largely consistent with textbooks and most studies (Vinay *et al*., 2007; Scheriner, 1991). Several examples are stated:

The pure stones were seen in 53.4% of our patients, which is near the number reported by Rayhan *et al.* (2003) (58.4%). In addition, calcium oxalate was the most common composition in our study (80.2%), uric acid, calcium phosphate, cystine and struvite were predominant compositions in 16.2%, 2.4%, 0.6 and 0.4% of patients, respectively. Rayhan *et al.* (2003) noted a high incidence of calcium oxalate stones (81.6%) as well as uric acid stones (9.6%), calcium phosphate stones (5.1%) and struvite stones (3.7%). Analysis of 69,949 stones in Japan showed that 79.4% were calcium oxalate and/or calcium phosphate stones, 7.4% were struvite, 5.2% were uric acid and 1.0% were cystine (Yoshida *et al*., 1990). Abdus Salam *et al.* (2004) found that majority of the stones in Saudi Arabia were composed of calcium (78%) followed by uric acid (19%) and phosphate stones 9 (3%). No cystine stone was found in this study. Almost similar findings were reported by Adriano *et al.* (2000) from Italy. He reported 70.1% calcium oxalate, 15.2% uric acid, 7.6% calcium phosphate, 1.5% cystine and 2.8% struvite in Italian patients. Baker *et al.* (1993) have reported these frequencies of the different stone types: calcium oxalate 68%; uric acid 17%; infection stones 12 and calcium phosphate 3%. Therefore, the relative incidence of calcium oxalate stones in our study was on the higher side as compared to other studies; uric acid and cystine stones are comparable and the only considerable difference is very low frequency of struvite in our study (Table 9).

In our patients with ages under 20 years, calcium oxalate and phosphate are the most common compositions (84%) and cystine is predominant in 3% of stones. These numbers are compatible with Kevan Sternberg's study which found 88% calcium based stones and 5% cystine; but predominance of uric acid in stones of 13% of our patients in this age group is a considerable difference. Struvite is also present in 7% of Kevan *et al.* (2005) patients and not found in our cases. Moreover, our results are greatly dissimilar to Cachat study in France. He reported below compositions in his pediatric patients with urolithiasis: struvite (35% of the cases), calcium-phosphate (25%) or calcium-oxalate (20%) (Cachat *et al*., 2004).

Maximum numbers of our cases were found in the summer months of August and June. These results suggest that there is a clear stone season in this area, corresponding to hot summer months. Baker *et al.* (1993) have reported significantly increased incidence of uric acid stones during summer and autumn and significantly decreased numbers of infection stones during spring and summer. In this study, no significant seasonal variation was observed with calcium oxalate or calcium phosphate stones. But surprisingly Atanasova has recorded a rather elevated frequency of spontaneous elimination in the winter months, which is irrespective of the type of stone (Atanasova *et al*., 1994). Kevan *et al.* (2005) reviewed his 12-year experience in pediatric stone disease and found that the seasons of presentation were fall in 41% of cases, summer in 24%, spring in 22 and winter in 13%. In our study, male patients show higher frequency of both calcium oxalate and uric acid stones in summer, but female patients have more calcium oxalate stones in autumn and more uric acid stones in winter. Rather consistently, Parks *et al.* (2003) found marked gender difference in the timing of stone risk as dual summer calcium oxalate and uric acid high risk in men and calcium oxalate in early winter in women.

There is no relation with Ramadan fasting in our study; this is consistent with study of Al-Hadramy in Arabia (1997). Moreover, Basiri *et al.* (2004) were compared the prevalence of renal colic in Ramadan with other months of the lunar year and found that there was also no significant difference between frequency of admissions in Ramadan and mean admission during cold half of the year. Mean admission in warm seasons were significantly higher than Ramadan.

This study confirms that calcium oxalate stones are the most common stone type, which is in accordance with studies from industrialized countries. A remarkably low frequency of struvite composition along with lower numbers of large staghorn stones are noted in our patients and especially those below 20 years old, which reflect low contribution of urinary tract infection in developing of urolithiasis in this population.

Hussain *et al*. (1990) had described characteristics of vesical calculosis in poor people of Northeast Bombay: Prevalence of bladder stones in children was high; all stones were of the mixed type; and ammonium urate was present in the majority of stones. These features aren't compatible with our results.

Several remarkable findings were noted in our study: very high frequency of calcium based stones, which is predominant composition in 82.6% of patients; very low contribution of ammonium urate, which is present only in 1.8% of patients and is predominant composition only in 0.1% of patients; high proportion of pure stones; and fewer children in our patients (2.5% are under 20 years old). With attention to these data, we may conclude that Ardabil province of Iran has passed the so called stone wave, i.e. the chronological changes in urolithiasis with increasing westernization of life-style and industrialization. It seems that affluence has spread to all social classes and with it the tendency to eat rich food in large quantities.

Our M/F ratios in different stone types are significantly different from other studies and the ratio is notably high in struvite; but we have not any explanation for it. Similarly, the difference of M/F ratio in different age groups is not consistent with other studies.

The exact pathogenesis of urolithiasis is not known. A number of promoters, inhibitors and predisposing factors can contribute to the development of stone formation. From the above results it appears that nutritional and environmental factors play a role in the pathogenesis of urolithiasis is Ardabil.

High protein intake along with the low water, oxalate rich diet like rice, tomatoes, salad and Coca-Cola group of drinks may play contributory role in the formation of calcium oxalate stones. Again high animal protein consumption and affluence may be one of the reasons of uric acid stone formation. The relatively decreased incidence of infective stones in our study may be due to the better health conditions and high standard of living.

The role of high calcium and magnesium content in the drinking water is not clear, but Torres *et al*. (1981) reported that at least it can pose problems for the urolithogenic prophylaxis in some patients.

CONCLUSION

In conclusion, there was a high prevalence of calcium oxalate and especially uric acid stones. Therefore, these compositions should be kept in mind when encountering stone patients in Ardabil, particularly for the prevention and prophylaxis of stone recurrence.

This study emphasizes the importance of stone analysis and extensive biochemical investigations in patients with urolithiasis, in order to avoid recurrence and potential progression towards chronic renal failure.

There are two forms of stone analysis: qualitative and quantitative. The most sensitive, specific and preferred method is quantitative analysis. Various physical methods of analysis may be used to determine the mineral composition of a stone sample, including optical crystallography, infrared spectroscopy and X-ray diffraction analysis (Ulrich *et al*., 1996). We performed semi-quantitative mineral analysis using chemical reagents. The chief limitation of the present study was the use of only one method for stone analysis. Preferably, stone analysis should have also been conducted by one or more additional methods, such as polarizing microscopy or X-ray diffraction analysis.

Finally, it is stated that nutritional and environmental factors seem to play a role in stone formation. Hence dietary intervention on a large scale and health education in this regard may be helpful on the preventive side. However, the concurrence of a genetic predisposition seems to be crucial for calcium stone formation.

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