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Evidence suggesting that high intake of fluoride provokes nephrolithiasis in tribal populations

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Abstract The present study was designed to evaluate the role of fluoride in urolithiasis in humans. Two areas were selected for this purpose, a fluoride endemic area (EA) and a fluoride non-endemic area (NEA). The prevalence of uroliathiasis was 4.6 times higher in EA than in NEA. Furthermore, the prevalence was almost double in subjects with fluorosis than without fluorosis in the endemic area. No relationship was observed between urolithiasis and the duration of fluorosis. The fluoride levels in drinking water ranged from 3.5 to 4.9 ppm in EA and subjects from this area excreted more fluoride. A comparison of normal subjects (NS) from EA and NEA revealed that endemic subjects tend to have slightly higher mean serum thiobarbituric acid reactive substance (TBAR) levels and excrete more oxalate and fluoride than their non-endemic counterparts. The urinary stone formers (SF) from the two areas showed a similar tendency, though again the difference was not significant. Citrate excretion in SF was almost normal in the EA, but NEA SF had significantly lower excretion levels. Urinary stones from endemic patients had higher fluoride, oxalate and calcium levels than those from non-endemic patients. In vitro studies suggested that fluoride did not influence the heterogonous mineralization of calcium oxalate. In conclusion, the data suggest that fluoride in vivo may behave as a mild promoter of urinary stone formation by (a) excretion of insoluble calcium fluoride, (b) increasing oxalate excretion and (c) mildly increasing the oxidative burden.

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Introduction

Fluoride is an essential element for human life. Human, animal and explant studies have shown that this element participates in the mineralization processes of bone and teeth [32], cementing crystals more strongly and thereby giving them more strength and hardness [7, 34]. In optimal concentrations fluoride renders protection against dental caries [14] but a far more serious global health problem involves fluoride toxicity leading to fluorosis due to high fluoride consumption. This is associated with several pathologies [12, 50]. Fluorosis has been reported both from developed and developing countries. In India over 25 million people from 15 states consume high levels of fluoride in water, ranging from 2 to 20 ppm [1, 49]. Rajasthan is one of the five worst affected states of India. In all 32 districts of this state, fluoride is in excess of the permissible limit (>1.5 ppm) in many localities, and in 18 districts, excess fluoride is a serious cause of fluoride toxicity. This is estimated to cause suffering in 3 million people residing in 9,741 villages and 6,819 habitations [31].

Experimental studies indicate that kidneys have excellent fluoride tolerance due to their efficient excretory mechanism. Changes in kidneys due to fluoride in the short term are expected only if its concentration in food and water combined is >120 mg/l, which is not encountered in practice. Whether long-term, high fluoride intake, as encountered by the population of Rajasthani (2–20 ppm) , can adversely affect the urinary tract or urinary milieu is a matter of debate. Anasuya [2] has demonstrated that high fluoride intake may lead to bladder stones in rats and Anasuya and Rao [3] noted increased mineralization in vitro. A high prevalence of urinary stones in Europe has been noted in a fluoride endemic area (EA) [48]. However, some workers have

not found any relationship between fluoride intake and bladder stones [46] and still others report it to be an inhibitor of stone formation [15]. The presence of fluoride in human urinary stones has been demonstrated and Machov [19] concluded that it is a universal constituent of urinary stones; however, whether its presence is adventitious or inherent is not clear. Various aspects of this problem are under study by our group and in this paper the prevalence of urinary stone disease, along with possible etiological factors are presented from a EA and fluoride non-endemic area (NEA) from the Udaipur region of India. A plausible mechanism for the involvement of fluoride in urolithiasis is also suggested.

Materials and methods

A total of 18,706 tribal people were personally interviewed to ascertain the prevalence of urinary stone disease. Only patients with a proven history were marked as "stone formers" (SF). Recurrence of the disease was defined as the appearance of fresh stones after the diagnosis of the first episode in either kidney or appearance of a new stone after previous removal or spontaneous voiding. Seventeen SF (renal-9, ureteric-5, vesical-3) were detected in NEA and 62 (renal-43, ureteric-14, vesical-5) were detected in EA. Out of these, 10,436 (male/female ratio – 1.39:1) and 8,270 (male/female ratio 1.1:1) were from NEA and EA, respectively. The number of children <12 years was 2,325 and 2,080 in the respective populations. Examination of 1,704 people from the endemic area revealed 840 with overt symptoms of fluorosis. Spot collections of urine and blood were carried out approximately 4 h after the morning meal. The fluoride levels in drinking water and urine were measured using the ion electrode method [31]. The urinary pH was measured by narrow range BDH papers. Calcium [13], oxalic acid [16], magnesium [22], GAGs [5], citric acid [28] and TBAR [6] were determined by standard procedures. Student's *t*-test was applied to evaluate significance.

The in vitro procedure of Jethi and Wadkins [18] was used to ascertain the influence of fluoride on heterogonous mineralization. Freshly slaughtered sheep collagen fibers, cut to approximately 10 mm, were obtained from the flexor tendon (metacarpel and metatarsal region), thoroughly cleaned and processed for collagen preparation as described in the procedure [18]. The incubating medium was prepared as follows: 1 ml of sodium chloride (10.5×10^{-2} M), 1 ml barbital buffer (17.5×10^{-3} M, pH 7.4), 1 ml calcium chloride (2.78 g $CaCl_2$/l to give a concentration of 25 μmol calcium/ml), 1 ml potassium oxalate (3.32 g $K_2C_2O_4$/l to give concentration of 20 μmol oxalate/ml), 1 ml sodium fluoride (containing 100 or 200 or 400 or 600 or 800 μg fluoride/l) and made to 25 ml with distilled water. The whole medium was thoroughly mixed and kept for 6 h. Thereafter, it was centrifuged and the supernatant was separated. Fifty milligrams of collagen fibers were then added and the flask was placed on a shaker for 24 h. Three-milliliter fractions were collected at 12, 18 and 24 h. They were either filtered through Wattman no. 3 filter paper or centrifuged at 1000 *g* for 15 min. The supernatant was used for the determination of calcium and oxalate concentrations. The decrease, if any, represented the uptake of calcium and oxalate for heterogonous nucleation.

For quantitative analysis, stones were washed, dried and crushed to a powder. Of this powder 100 mg was treated with 25 ml of aquaregia (1:3 HNO_3:HCl) and heated for 1–2 h until 1–2 ml of residue was left. This was dissolved in 100 ml of warm distilled water and then centrifuged at 1000 *g* for 10 min. The supernatant was used for the determination of calcium, magnesium and phosphorous by the methods stated above. For oxalate and ammonium estimation 10–50 mg of urinary stone was weighed and sulfuric acid was added to give a concentration of 5 mg/ml acid. This was centrifuged and the supernatant was used for the determination of oxalic acid [16] and ammonium [22]. For uric acid, urinary stone powder was mixed with lithium carbonate in the ratio 4:3 and dissolved in distilled water. It was then centrifuged and the supernatant was used for the estimation of uric acid by the method of Caraway as described for serum [9].

Results

In the NEA the prevalence of urinary stone disease in the tribal population was 163/100,000. Males were 11.5 times more often affected than females. In EA the prevalence was 750/100,000. Furthermore, the prevalence was 1,071/100,000 in patients with fluorosis and 578/100,000 in patients without fluorosis. Prevalence was higher in males than females in all categories. The recurrence of the disease was nearly double in EA compared to NEA (Table 1). No relationship was observed between prevalence of urolithiasis and duration of fluorosis (Table 2). The various clinical symptoms in fluorotic subjects were noted (Table 3). Kidneys are almost the sole excretors of absorbed fluoride and absorption is dependent on intake Table 4. In the EA, normal subjects (NS) had higher fluoride and oxalate excretion and lower magnesium excretion than NS from NEA whereas citrate and TBAR excretion were comparable. The SF from EA and NEA showed a similar picture except that citrate excretion was higher in the former. Serum TBAR levels were higher in SF of both the groups compared to NS, and SF from EA tended to have higher levels (3.8 ± 1.5 nmol/ml) than SF from NEA (3.2 ± 1.3 nmol/ml) (Table 5). The result of in vitro studies indicated that fluoride had no influence on heterogonous mineralization (Table 6). Urinary stones from EA had significantly higher levels of fluoride than from NEA (Table 7).

Discussion

Prevalence of urolithiasis is high in many parts of India [10, 17, 23, 35, 39, 42] including Rajasthan [26, 27, 43, 44]. The involvement of possible etiological factors in this disease has been reported from time to time [4, 25, 29, 38, 39, 40, 41, 45]. In an earlier study, no relationship was observed between fluoride in drinking water and the prevalence of urinary stone disease in a NEA [36]. However, a recent, preliminary study by our group noted a high prevalence of urolithiasis in EA. The present, extended study supports this finding and further suggests that chronic fluoride consumption increases the risk of urinary stone formation by altering the urinary profile and mildly increasing the oxidative environment.

A door to door survey for urolithiasis among non-tribal people in this region showed a prevalence of 310/100,000, with a male/female ratio of 5.5:1 [43]. On the other hand, this study, as well as other studies from this region [30, 43, 45], indicate that the prevalence is much lower among tribal people. Environmental provocations

Table 1 Prevalence of urinary stone disease in a fluoride endemic and a fluoride non-endemic area. The number of recurrent patients is given in parentheses

Category	Number of people surveyed			Number of patients			Prevalence/100,000			Male/female ratio	Recurrence (%)
	Total	Male	Female	Total	Male	Female	Total	Male	Female		
Non-endemic area	10,436	6,067	4,369	17	16	1	163	264	23	11.5:1	17.6
Mean age (years)				33.1 ± 9.2 (3)	33.5 ± 9.4 (3)	42					
Endemic area	8,270	4,270	4,000	62	44	18	750	1,030	450	2.3:1	35.5
Mean age (years)				31.7 ± 10.7 (22)	30.0 ± 9.7 (18)	35.7 ± 11.9 (4)					
Subjects surveyed for fluorosis											
Total	1,704	854	850	14	10	4	–	–	–	–	–
Mean age (years)	–	–	–	35.0 ± 9.5	33.1 ± 5.6	39.7 ± 14.2	–	–	–	–	–
With fluorosis	840	431	409	9	7	2	1071	1,624	489	3.3:1	–
Mean age (years)	–	–	–	36.7 ± 11.2	33.4 ± 6.4	48.0 ± 16.0	–	–	–	–	–
Without fluorosis	864	423	441	5	3	2	578	709	453	1.6:1	–
Mean age (years)				32.0 ± 3.3	32.3 ± 3.1	31.5 ± 3.5					

are known to enhance nephrolithiasis [4, 11] and Barjatiya and Singh [4] have already reported higher prevalence of nephrolithiasis among tribal people working in a mining environment in this region. In the current study we observed a 4.6-times higher prevalence in EA than in NEA. The role of fluoride as a urinary stone risk factor is further supported by the finding that prevalence in fluorotic patients was double (1,071/100,000) that found in non-fluorotic patients (578/100,000). However, no relation was found between the duration of fluorosis and the prevalence of urolithiasis. This can be attributed to its multifactorial etiology.

The main contributor to fluoride intake in humans is drinking water and in Rajasthan water fluoride levels in EA are in the range of 2 to 20 ppm [20, 50]. In the EA under study, fluoride levels ranged from 3.5 to 4.9 ppm. According to the fluoride map of Rajasthan, the populations of Udaipur and Dungarpur districts suffer from a relatively moderate degree of fluoride toxicity [21]. Since the concentration of urinary constituents is the most important determinant of urinary stone formation and growth, urine chemistry of tribal NS and SF from EA and NEA was examined for stone promoters and inhibitors. In NEA the urinary profile of NS was comparable to other studies [4, 26, 45]. The SF in this population, as in earlier studies [43, 45], showed three important differences from NS, i.e., higher excretion of oxalate and calcium and lower excretion of citrate. However, the level of calcium does not appear to be an important contributing factor. In the EA both NS and SF had either significantly higher oxalate excretion or tended to have higher excretion. Citrate excretion was normal.

Subsequently, NS and SF from EA were compared with their counterparts in NEA. The NS from EA showed two important differences from NS from NEA, i.e., significantly higher oxalate and fluoride and lower magnesium excretion. The SF from these two areas also showed similar trends, though oxalate and magnesium did not attain the level of significance. However, an additional change in SF from EA was the normal level of citrate excretion. Citrate is undoubtedly a good inhibitor of urinary stone formation and plays multiple roles in the prevention of calcium lithiasis [24]. However, its relative potential as an inhibitor is expected to lose much of its importance in this population which has low calcium excretion. The NS in EA showed a tendency to have slightly higher TBAR levels and SF in both groups had slightly higher levels than their NS counterparts. These data, and our unpublished data on animals, suggest that fluoride slightly increases the oxidative environment in situ. Higher oxidative load in SF compared to NS has been reported earlier, also from Rajasthan [8]. An enlarged oxalate pool in vivo can lead to oxidative stress [47] and this stress can damage or kill the renal epithelial cells [33] which can then serve as a site or nidus for the formation of a urinary stone.

There are a number of controversies on the behavior of fluoride in the context of lithogenesis:

Table 2 Distribution of symptomatic subjects according to the duration of the fluorosis

Duration of fluorosis	Patients with symptoms of fluorosis						Total stone formers ($n=9$)
	Total (840)		Male (431)		Female (409)		
	n	%	*n*	%	*n*	%	Ratio[a]
1 year	276	33	121	28	155	38	–
2–5 years	496	59	271	63	225	55	0.22
6–10 years	50	6	29	7	21	5	0.55
> 10 years	18	2	10	2	8	2	0.22

[a] Represents number of patients with different duration of fluoruosis to total number patients

Table 3 Distribution of patients with different symptoms of skeletal and non-skeletal fluorosis from the Dungarpur block

Category	Number of patients	Percent (%)
Skeletal fluorosis:		
Pain in limbs	25	3
Pain in joints	576	67
Pain in neck and backbone	590	69
Inability to perform routine domestic work	100	12
Non-skeletal fluorosis:		
Loss of appetite	222	26
Pain in abdomen	205	24
Flatulence	105	12
Constipation	102	12
Depression and nervousness	257	30
Increased thirst	100	12
Tingling sensation in fingers and toes	16	2
Increased frequency of urination	90	11
Painful skin rashes	8	1

a. Why did Teotia et al. [46] not find any relationship between fluorosis and bladder stone disease in children whereas others have noted a high prevalence in humans and in experimental animals with a high fluoride intake from drinking water [48].
b. Why Anasuya [2] found fluoride to be a promoter in rats whereas Herring et al. [15], found it to be an inhibitor.
c. Why some in vitro studies found fluoride to be a promoter [3] whereas others found it to be unrelated to the risk of urinary stone formation [36], and
d. Why the present study assigns fluoride the role of a promoter of urinary stones.

The conclusions derived by Teotia et al. [46] are suspect because they neither studied bladder stone disease nor examined the prevalence and the urine chemistry of NS and SF, in EA. Herring et al [15] were precipitate in concluding that fluoride is an inhibitor because they examined neither the frequency of calcium oxalate crystals nor the size of crystals nor urinary oxalate which are prerequisites for their conclusion. They also missed the point that fluoride is a highly reactive anion and quickly forms calcium fluoride which is insoluble in water. In all probability, the lower calcium content in the kidneys of their rats receiving high fluoride is due to the lower availability of calcium for absorption. This is because calcium fluoride precipitates in the intestine and would not be available for absorption. For the same reason, there would have been diminished availability of calcium in their in vitro experiments for the formation of calcium oxalate. A close scrutiny reveals that the primary cause for bladder stones in Anasuya's experiment was diet related and not due to fluoride because 25% and 33% of rats respectively developed bladder stones on low and high calcium control diets during the 10-week period of study. Fluoride only accentuated the risk of stone formation.

In our study, the stones from endemic SF have significantly higher fluoride content and calcium content also tended to be higher. On the basis of his observations Machov [19] concluded that fluoride was a permanent

Table 4 Fluoride level in drinking water and urine [recommended level of fluoride in water by the Indian Council of Medical Research (1975) is 1.0 ppm]

Name of village	Drinking water			Urine	
	In ppm	% of wells having < 1.0 ppm	ppm	Mean ± SD	% of subjects having < 0.5 ppm
Endemic area:					
Khara Samor	3.70	–	0.25–2.30	1.10 ± 0.58	18
Vasunderbadi	3.50	–	0.41–2.60	1.26 ± 0.60	14
Kankri	4.40	–	0.60–3.40	1.58 ± 0.78	0
Ghata	4.90	–	1.30–5.50	2.41 ± 1.10	0
Gahunwada	3.60	–	1.30–9.90	2.29 ± 1.89	0
Non-endemic area:					
Salai	0.50	100%	0.30–0.95	0.60 ± 0.49	50

Table 5 Urine chemistry and serum TBAR levels along with statistical comparison of normal subject (*NS*) and stone formers (*SF*) residing in an endemic area (*EA*) and a non-endemic area (*NEA*). The number of subjects is given in parenthesis

Urine	EA		NEA	
	NS	SF	NS	SF
Calcium (mg/gCr)	101 ± 42 (15)	139 ± 80 (14)	99 ± 38 (16)	132 ± 18[c] (17)
Magnesium (mg/gCr)	54 ± 24 (80)	55 ± 19 (20)	[c]87 ± 28 (60)	70 ± 41[b] (24)
Oxalate (mg/gCr)	36.1 ± 15.0 (80)	44.2 ± 24.6[a] (20)	[d]23.8 ± 16.5 (60)	34.3 ± 13.5[b] (24)
Citrate (mg/gCr)	565 ± 156 (80)	435 ± 188[c] (20)	558 ± 176 (60)	[b]305 ± 206[d] (24)
TBAR (μmol/gCr)	4.4 ± 1.8 (80)	4.6 ± 1.5 (20)	4.2 ± 1.5 (60)	4.2 ± 1.7 (24)
Fluoride (mg/gCr)	2.5 ± 1.6 (80)	2.3 ± 1.2 (20)	[d]0.9 ± 0.8 (60)	[d]1.1 ± 0.6 (24)
Serum TBAR (nmol/ml)	2.3 ± 1.04 (26)	3.8 ± 1.5[b] (8)	1.9 ± 1.1 (40)	3.2 ± 1.3[c] (8)

Statistical significance, *P* value a < 0.1, b < 0.05, c < 0.01 and d < 0.001
P value on the right side indicates a statistical comparison between NS and SF from the same area (EA AND NEA)
P value on the left side indicates a statistical comparison between NS (EA) and NS (NEA) and between SF (EA) and SF (NEA)

Table 6 Effect of fluoride on heterogonous mineralization of calcium oxalate in vitro (values are mean ± SD μmol in soluble fraction)

	Fluoride (μg/l)					
	Control	100	200	400	600	800
Calcium						
12 h	16.9 ± 1.8	16.6 ± 2.3	16.6 ± 1.6	17.4 ± 2.4	16.5 ± 2.1	16.7 ± 2.5
18 h	15.8 ± 1.5	15.5 ± 1.2	16.0 ± 2.3	16.2 ± 2.5	15.9 ± 1.6	16.2 ± 2.2
24 h	15.0 ± 1.7	15.2 ± 2.1	15.5 ± 1.8	15.7 ± 1.9	15.4 ± 1.5	15.8 ± 1.6
Oxalate						
12 h	1.5 ± 0.5	1.8 ± 0.3	1.4 ± 0.8	2.4 ± 0.9	1.6 ± 0.5	1.8 ± 0.6
18 h	0.6 ± 0.4	0.6 ± 0.5	0.9 ± 0.5	1.1 ± 0.4	0.8 ± 0.5	0.9 ± 0.6
24 h	0.1 ± 0.2	0.3 ± 0.2	0.6 ± 0.4	0.8 ± 0.3	0.2 ± 0.1	0.6 ± 0.5

Table 7 Quantitative composition of urinary stone

Parameters	Endemic area ($n = 8$)	Non-endemic area ($n = 35$)
Protein (mg/100 g)	6.7 ± 2.0	6.3 ± 3.1
Calcium (mg/100 g)	15.1 ± 2.7	12.1 ± 8.5
Magnesium (mg/100 g)	2.5 ± 0.7	1.9 ± 1.9
Oxalate (mg/100 g)	27.0 ± 4.0	20.3 ± 10.3
Phosphate (mg/100 g)	7.0 ± 1.5	6.3 ± 4.6
Ammonium (mg/100 g)	6.5 ± 1.4	8.0 ± 8.6
Uric Acid (mg/100 g)	5.8 ± 1.0	5.8 ± 4.2
Fluoride (μg/100 mg)	295 ± 72	86 ± 41*

* $P < 0.001$

constituent of urinary stones. He also suggested that it is an active constituent. Our data are in conformity with his findings although we do not believe that fluoride plays an active role. Our view is also supported by the work of Anasuya [2], who found that weights of stones in rats with low and high fluoride intake were similar. Most likely, the insoluble calcium fluoride in water is fortuitously incorporated into the stone.

While Anasuya and Rao [3] noted fluoride to be a promoter in their in vitro studies, our data do not support their observations. The increased deposition of calcium on tendons in their study may be due to deposition of water insoluble calcium fluoride. We did not find any change in calcium uptake when this possibility was eliminated.

Taking into consideration all of the data from our study and from the studies of others, we believe that the chronic intake of fluoride increases the risk of urinary stone formation. The proposed mechanism of fluoride participation in lithogenic processes in the tribal population studied could be as follows: The Rajasthani population in general, including tribal people, suffer from a high excretion of oxalate, mainly due to defective nutrition [37, 38, 43, 45]. Fluoride may be indirectly increasing oxaluria by enhancing the absorption of oxalate from the intestine due to low availability of calcium as part of the intestinal calcium is precipitated as calcium fluoride. Oxalate in –vitro, as well as in –vivo, increases oxidative load [33, 47] which may injure or kill renal epithelial cells, providing an opportunity for urinary crystals to attach to the injured site or to be deposited on dead epithelial cells which serve as a nidus. Fluoride may also be synergistically acting with oxalate to potentiate the oxidative environment. Fluoride is also thought to possess the inherent property of being able to bind crystals strongly, which gives more hardness to bone [7]. It is, therefore, logical to presume that the formation of a urinary stone will occur when these conditions overwhelm the inhibitory forces present.

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