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LOP-SIDED REDOX IS RISK FACTOR IN ETIOPATHOGENESIS OF NEPHROLITHASIS IN SOUTHERN RAJASTHAN

Biochemistry

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ABSTRACT

Slightly weak antioxidant status and raised oxidative stress is suspected in renal disorders including renal stone disease. Hyper-oxaluria and hypocitraturia are also reported as risk factors in renal stone disease. A possible collusion of oxidative stress, hyper-oxaluria and hypo-citraturia in etiopathogenesis of nephrolithiasis has been suggested.

Objective- To examine whether imbalanced redox and hyperoxaluria, possess a risk in nephrolithiasis.

KEYWORDS

Nephrolithiasis, Nutrient antioxidants, Enzyme antioxidants, Oxidative stress, Hyper-oxaluria.

INTRODUCTION

Prevalence of urolithiasis is very high in this region,[1] Zinc (Zawar) and Rock Phosphate (Matoon) mines have still higher prevalence. Many parts of Rajasthan, including some areas in southern region suffer from high content of fluoride in food and water. High incidence of urolithiasis, implicating fluoride is also reported.[2]

An ICMR funded project revealed a prevalence of 256.9/1,00,000 population; incidence of 3.4/yr/1,00,000 population, recurrence rate of 27% and male female ratio of 6:1 (Singh, P.P. et all, 1990 ICMR Final Report). Hyper-oxaluria and hypo-citraturia were prominent features and they continue to remain so. Raised oxidative stress (OS) and weak antioxidant defence, suggesting lop-sided redox, in renal disorders is also reported from here.[3] Hyper-oxaluria, hypo-citraturia and raised OS are potential risk factors in the genesis of nephrolithiasis.[4]

Aerobic cells are electron sink. The concentration of electron is maintained and modulated by oxidants [reactive oxygen species (ROS) and reactive nitrogen species (RNS)] and their removal by antioxidants (dietary and endogenous). There is always a little excess of oxidants. It is referred as oxidative stress (OS) or Redox. There is an intermittent subtle fluctuation in OS (redox shift). These redox flexes impel numerous energy driven physiological functions such as proliferation, differentiation, apoptosis, autophagy, mitophagy, and many others.[4-5]

Nevertheless, low or raised OS, caused by internal or external stimuli leads to physiological disturbances and diseases. Low OS is rare, causes diminished signaling and decrease in vital protein disulphides. It is raised OS which is a cause of worry for clinicians and has been implicated in more than hundred diseases.[6-13]

Presently there is putative role of OS in chronic diseases including renal stone disease (RSD).[4,6,8] However raised OS is never a primary cause of lithogenesis. Clinical, animal model and cell culture studies have explicitly shown that raised OS does not induce calcium oxalate deposition in renal tissue nor calcium oxalate or calcium phosphate crystalluria nor nephrolilthiasis. On the other hand, increased oxalate concentration in renal tissue raises OS and then these in concert lead to stone formation. If, this hypothesis is true, then hyper-oxaluria intuitively should be accompanied with raised OS in RSD. Our observations in the present series strengthen this presumption.

First, we have examined OS, nutrient antioxidant status and enzyme antioxidant activity in 90 normal subjects (NS) and 90 RSD patients and then compared with our two previous series reported earlier. Raised OS and slightly feeble antioxidant status has been observed in all the three series. Second, we have extended our studies in this series to include "Total Antioxidant Power" of plasma by FRAP method,[14] (Ferric Reducing Antioxidant Power) and reduced glutathione (GSH). GSH is a master molecule in cellular antioxidant arsenal.[15] Additionally we have also examined xanthine oxidase (XO) which is a pro-oxidant enzyme. XO is a downstream product of xanthine-oxido-reductase with major function of generating free radicals. It increases in inflammation and injury.[16]

MATERIAL AND METHODS

Selection of normal subjects and renal stone disease (RSD) patients

The normal subjects were healthy persons belonging to middle or upper middle class family (males-50 and females-40) and renal stone disease (RSD) patients were radiologically proven stone patients (stones in kidney or ureter) (males-50 females-40). The patients were selected from different wards of M.B. General Hospital or attending the OPD.

$P < 0.05$ was considered as significant.

Collection of blood sample and Methods

Fasting blood samples of both normal subjects and RSD patients were collected in clean syringes. 4 ml blood was transferred in plain vials and 3ml containing EDTA. Serum was separated from blood collected in plain vials. Plasma and RBC were separated from blood collected in EDTA vials by centrifugation at 1500 rpm for 15 mts. Serum was used for determination of vitamins A[17],E,[18] and C[17] and beta-carotene[17], Plasma was used for determination of Total Antioxidant Capacity by FRAP method.[14] RBCs were washed thrice with normal saline and then lysed in distilled water in the ratio of 1:3. Hemolysate was used for determination of superoxide dismutase (SOD)[19] catalase (cat)[20], glutathione peroxidase (GPx)[21] xanthine oxidase[22], reduced glutathione (GSH)[23] and OS (TBARS).[24] Standard methods were used for the determination of all parameters.

24 hr. urine samples of 42 male normal subjects and 42 male renal stone formers were collected in 2.5 Lit. brown bottles using chloroform as preservative, collected urine samples were thoroughly shaken. 10 ml sample was collected in thoroughly clean plain test tubes. It was used for the determination of calcium,[25] and citric[26] acid. The remaining urine sample was mixed with100 ml 1-N-HCl and thoroughly shaken to dissolve the calcium oxalate crystals, if any. It was used for the determination of oxalic acid by chemical method of Hodgkinson and Williams.[27]

Unpaired student test was used to assess the difference and Pearson Correlation Coefficient (r) to assess relationships.

RESULTS

The results of the nutrient antioxidants and enzyme antioxidants in NS and RSD patients are given in tablets IA and 2A.

Table-1A Nutrient Antioxidants in Normal Subjects and Renal stone Formers : Present Series

Parameter	Normal Subjects		Renal Stone Formers	
	Males (n=50)	Females (n=40)	Males (n=50)	Females (n=40)
Retinol mg/dl	26.62 ±2.80	23.67 ± 3.26	25.81 ± 2.66	26.15 ± 3.35
Beta-Carotene mg/dl	180 ± 24	152 ± 25	182 ± 26	198 ±32 *
Alpha-Tocopherol mg/dl	1.28 ± 0.41	1.04 ± 0.50	0.78 ± 0.44 *	0.76 ± 0.36 *
Ascorbic Acid mg/dl	1.43 ± 0.32	1.28 ± 0.45	0.71 ± 0.38 *	0.82 ± 0.22 *

Table-2A: Plasma Total Antioxidant Power (PRAP method), erythrocyte Reduced Glutathione, Xanthine Oxidase and TBARS Level : In Present Series

Parameter	Normal Subject		Renal Stone Formers	
	Males (n=60)	Females (n=40)	Males (n=50)	Females (n=40)
Total Antioxidant Power le mol /dl	890 ± 115	924 ± 94	648 ± 104*	595 ± 88*
Reduced glutathiroric mg/dl	78 ± 14	74 ± 12	60 ± 15 *	59 ± 12*
Xanthine Oxidase m mol uric acid/min	517 ± 82	476 ± 65	922 ± 102*	970 ± 105*
TBARS Mol/ml	2.50 ± 0.35	2.51 ± 0.46	4.05 ± 0.59 *	3.85 ± 0.80*

The same parameters were earlier reported in 2002 and 1996 in the local population. To get first hand information for comparison, the data are tabulated in tables 1B and 2B.

The four consistent observations among renal stone formers in the three series are: a) relatively lower concentration of alpha-tocopherol and ascorbic acid (though not deficient), b) lower activity of SOD c) raised activity of Cat d) raised level of OS. The GPx activity has been examined in only two series and did not show any difference between RSD patients and NS. The lower activity of alpha-tocopheral and ascorbic acid should obviously be due to lower intake of these two vitamins in diet.

Table-1B Serum Nutrient Antioxidants Reported From Our Laboratory in 2002 and 1996

Parameter	Reported in 2002		Reported in 1996		
	Normal Subject (50-60ys age)	Renal Stone Former (50-60ys age)	Normal Subject	Renal Stone Formers Males	Renal Stone Former Females
Retinol mg/dl	23 ± 6	21 ± 6	22.9 ± 8.9	20.16 *	17.80 ± 060 *
Beta-Carotene mg/dl	196 ± 79	146 ± 55 *	176 ± 72	130 ± 53 *	190 ± 69 *
Alpha-Tocopherol mg/dl	0.95 ± 0.19	0.86 ± 0.16 *	0.88 ± 0.17	0.86 ± 0.17 *	0.80 ± 0.16 *
Ascorbic Acid mg/dl	1.07 ± 0.20	0.86 ± 0.17 *	10.4 ± 0.22	0.98 ± 0.18 *	0.90 ± 0.18 *

Table-2B Total Antioxidant Power, erythrocyte Reduced Glutathoric, Xanthine Oxidase and TBARS Level in Serum : Reported in 2002 and 1996 From This Laboratory

Parameter	Reported in 2002		Reported in 1996		
	Normal Subjects	Renal Stone Formers	Normal Subjects	Renal Stone Formers	
				Males	Females
Total Antioxidant Power le mol /L	-	-	-	-	-
Reduced glutathiroric mg/dl	-	-	-	-	-
Xanthine Oxidase	-	-	-	-	-
TBARS Mol/ml	2.56 ± 0.76	3.35 ± 0.77	2.42 ± 0.66	3.50 ± 0.81	3.30 ± 060

All the antioxidant enzymes viz SOD, Cat and GPx are inducible enzymes thereby implying that their activity can be stretched on either side. It makes extremely difficult to speculate the exact reason for lower activity of SOD in RSD patients. The only plausible reason could be release of some metabolic inhibitor in RSD patients. Only future studies can resolve this issue. Tissues can convert O2 to H2O2 but at a very slow rate. SOD enhances this reaction by 10,000 fold. As such, even the lower SOD levels, as observed in our series should be fair enough to dismutate O2 at desired speed. The Cat is extremely fast in converting H2O2 to H2O and O2 in presence of oxygen. The raised Cat activity in RSD patients is indicative of faster synthesis of H2O2.

The notable feature is consistently raised TBARS in RSD patients in all the three series. It points out that RSD patients face relatively more oxidized environment, i.e. disturbed redox balance. Though the rise in erythrocyte TBARS level is significant but mild to moderate. It is almost similar in all the series Overall these results indicate a chronic mild disturbed redox, status. Xanthine oxidase level in erythrocytes indicates its level, in vivo. Its level was significantly raised in RSD patients (males 922 ± 102, females 970 ± 105) compared to NS (males 517 ± 82 females-545 ± 69/mmole uric acid), which suggests that it may possibly be involved in raising OS in kidney and elsewhere in the body and may be accomplice with oxalate in the genesis of nephrolithiasis. To best of our knowledge, we are reporting it for the first time. Further studies are needed to authenticate this finding.

Table-3A Erythrocyte Antioxidant Enzymes in Normal Subject and Renal Stone Formers

Parameter	Normal Subject		Renal Stone Formers	
	Males (n=50)	Females (n=40)	Males (n=50)	Females (n=40)
SOD units/ml	3.04 ± 0.46	2.99 ± 0.58 *	2.09 ± 0.50 *	2.01 ± 0.65 *
Gpx units/ml	5.86 ± 0.72	6.22 ± 0.80	6.91 ± 1.02	5.73 ± 0.88
Catalase le mol H2O2/mg/sec	7.72 ± 0.75	7.44 ± 0.76	10.44 ± 1.05 *	12.24 ± 1.08 *

Table-3B Erythrocyte Antioxidant Enzymes in Normal Subject and Renal Stone Formers

Parameter	Reported in 2002		Reported in 1996		
	Normal Subjects 50-60 yr age	Renal Stone Farmers 50-60 yr age	Normal Subjects	Renal Stone Farmers	
				Males	Females
SOD units/ml	2.07 ± 0.71	1.80 ± 0.54 *	2.82 ± 0.76	2.1 ± 0.6*	2.05 ± 0.50*
Gpx units/ml	6.50 ± 0.90	6.46 ± 0.82	-	-	-
Catalase le mol H2O2/mg/sec	6.97 ± 207	9.86 ± 3.07	0.17 ± 2.16	10.3 ± 3.2*	9.1 ± 3.0*

The methods measuring "Total Antioxidant Power" of plasma are considered to be more informative as they provide total strength of non-enzymatic strength of antioxidant team (nutrient antioxidants and dietary antioxidants in diet and endogenous non-enzymatic

antioxidants.). The FRAP method used in this study offers a putative index of antioxidant or reducing potential of plasma. (Table-3) The suggested normal range is 500-1500 μ mol/lit. In present series it was 890±115 in males and 835 ± 94 μ mol/lit in females. It was significantly lower in RSD patients (615 ± 142 μ /lit in male and 602 ± 108 μ mole/lit in female). The FRAP method however does not measure GSH levels, so it was also measured. It was fairly low in male and female RSD patients (P<0.05). Lastly, 24 hr urinary excretion of calcium, oxalic acid and citric acid and TBARS were measured in 42 male NS and 26 normo-oxaluric and 16 hyper-oxaluric RSD patients.

Simultaneously serum TBARS levels were also measured (Table-4). The notable features were raised calcium, oxalate and low citrate levels in urine even in normo-oxaluric RSD patients as compared to NS. Their excretions were enhanced further in hyper-oxaluric patients and interestingly, serum TBARS excretion showed similar trend. No significant relationship was observed between oxalate vs. TBAR levels and citrate vs. TBARS levels in urine.

Table-4: Erythrocyte TBARS levels and 24 hr urinary Excretion of Calcium, Oxalic Acid, Citric Acid And TBARS in 42 Male Normal Subject, 26 Normo-Oxaluric and 16 Hyper-Oxaluric Renal Stone Former

Parameter	Normal Subject (n=40)	Renal Stone Formers	
		Normo-Oxaluric (n= 26)	Hyper-Oxaluric (n = 16)
Erythrocyte TBARS n mol/ml	2.43 ± 0.26	3.92 ± 0.65*	4.88 ± 0.60**
Calcium (mg)	166 ± 26	194 ± 25*	265 ± 32**
Oxalic Acid- (mg)	22.6 ± 3.8	32.0 ± 3.33*	51.6 ± 6.2**
Citric Acid (mg)	6.12 ± 75	395 ± 81*	290 ± 85**
TBARS n mol/ml	358 ± 64	545 ± 78*	740 ± 75**

Normo-Oxaluric - Excretion <40.0 mg/24hrs.
AHyper-Oxaluric - Excretion > 40.0 g/24hrs.

DISCUSSION-

We are presenting here third series of data on redox regulatory factors (OS and antioxidants) in the local population of southern Rajasthan. The first series of data were presented by one of us (M.K.B.) in International Symposium on Urolithiasis and Renal Disorders in Dallas (USA) in 1996 including RSD. The data highlighted raised OS and weaker antioxidant defence in renal disorder patients including RSD, which suggested imbalanced redox a risk factor. The second series of observations were reported in 2002 on RSD in relation to age.[28] It re-stressed imbalanced redox due to raised OS and weaker antioxidant defence. Now a third series of observations, more comprehensibly studied and examined, are presented. Total antioxidant capacity reduced glutathione (GSH) and xanthine oxidase are new features Further for the first time, 24 hr urinary exertion of calcium, oxalate, citrate, TBARS along with erythrocyte TBARS level are examined in 42 male NS, 26 male normo-oxaluric and 16 male hyper-oxaluric RSD patients. The data are quite raveling as these provide new information in that XO may be involved in provoking OS, and that oxalate and OS in concert are probably involved in the genesis of calcium oxalate nephrolithasis. As far as we know, data on XO in RSD is being reported for the first time.

Three observations in this series require especial mention. First, the level of nutrient antioxidants in NS was almost comparable with two previous series. Second, the level of these antioxidants in RSD patients was also within normal range. Third, nevertheless level of alpha-tocopherol and ascorbic acid was significantly lower than NS.

Both alpha-tocopherol and ascorbic acid are very strong chain breaking antioxidants.[29] In simple terms these antioxidants donate hydrogen atom to free radicals to convert them into non-radials, thereby putting brake on the formation of new free radical species, however, in turn the antioxidant becomes a free radical. In cytosol, there is a team of antioxidants following hierarchy in which ascorbic acid is first one. Vitamin E intercalates in the cell membrane which is rich in poly- unsaturated fatly acids, especially arachidonic acid. These fatty acids are extremely prone to free radical attack. Alpha-tocopheral efficiently detoxifies free radicals snatching electron before they could damage cell membrane obviously in-turn alpha-tocopheral becomes a radical.

Ascorbic acid is extremely effective antioxidant in cytosol. It efficiently relieves alpha-tocopheral radical at the interface of cell membrane and cytosol. While alfa-tocopherol is ready to recycle, in turn now ascorbic acid becomes a radical. Then ascorbic acid radical passes on its electron progressively on a chain of antioxidants with slow release of energy at each step till the last radical has only ground level of energy and becomes harmless. Implicitly, this means that their concentration must be maintained at highest level of normal to get optimum antioxidant activity. (normal level of alpha-tocopherol-05-2.0mg/dl, ascorbic acid 0.5-2.0mg/dl). The results in this series, therefore, suggest that the level of alpha-tocopherol and ascorbic acid should be raised to achieve optimized antioxidant activity. This can be done either by improving diet or therapeutic supplement. Retinol and beta-carotene level are normal in RSD patients. But otherwise also both of these are very weak antioxidant in vivo in human body.

Presently, the measurement of "Total Antioxidant Activity" (TAA) in plasma is considered to be better index for non-enzymatic antioxidants level than measuring individual antioxidants. In this series it was measured by FRAP method (Ferric Reducing Antioxidant Power). This method is quite reliable but it does not measure reduced GSH level. Hence, GSH was also measured. The TAA was significantly and substantially lower in RSD patients. The best way to raise it is by supplementing alpha-tocopheral and ascorbic acid. The supplement of alpha-tocopheral and ascorbic acid is quite safe upto 1000 mg/day and 1500mg/day respectively.[30]

GSH is a versatile molecule and is the most potent non-enzymatic endogenous antioxidant.[15] Indeed, in human body it exists in two inter convertible forms viz reduced glutathione (GSH) and oxidized glutathione (G-S-S-G) in the ratio of 100:1. Glutathione buffer (GSH: GSSG) is key player for the maintenance of cellular redox homeostasis. A deficiency of GSH shows an upset in redox box, resulting in promotion or initiation of a large number of diseases. Low level of GSH has been implicated in eito-pathogenesis of RSD.[4]

In this series GSH level was significantly low in patients. Beside, potent antioxidant property some other important functions of GSH are : detoxification of endogenous and exogenous toxins of electophilic nature, maintaining thiol concentration of proteins, cysteine and other thiol compounds, storage of cysteine reserve in cells or interorgam transfer, synthesis of oesterogens, leukotriences and prostaglandins, conversion of RNA to DNA, maturation of iron-sulphur clusters in proteins, copper and iron transfer, transduction from environment to cellular transduction machinery, lastly and most importantly glutathione buffer also serves as biosensor for numerous redox driven reactions. Many of these functions are involved in free radical metabolism. The above statement underscores the importance of GSH in human body. Since GSH level is significant to low in RSD, it should be raised Several therapeutic options are available for this purpose including vitamin C or lipoic acid supplement.

Owing to necessicity to keep ROS and RNS within a range compatible to normal cell or organ, humans have also evolutionarily developed a number of modulating antioxidant enzymes to assist non-enzymatic antioxidants. The main players are SODs, Cats and GPXs. SODs are exclusive enzymes to dismutase superoxide anion to H2O2. (hydrogen per-oxide). H2O2 has comprehensive duty as signaling molecule but whenever in excess plays an invective role. Cats and GPx, maintain physiological concentration of H2O2 by detoxifying excess of it water and oxygen. Both these enzymes have distinctly divided roles. For example, Cats act at low concentration of H2O2 whereas GPx at high concentration. The level of these three enzymes are reported in this and earlier series. In first series GPx was not reported. SOD activity was significantly lower and Cat activity was higher in RSD patients in all the series. GPx, reported in two series, did not show any difference. It could be summarized from these data that SOD dismutated superoxide anion to release H_2O_2 and Cat. activity was raised to scavenge excess H_2O_2 . Indirectly the observations convey that superoxide anion concentration is raised. Cat activity as raised to scavenge H_2O_2.

Complicity, all the data on nutrient antioxidants, total antioxidant

strength, low GSH level, Nutrient and antioxidants low SOD and raised Cat level taken together suggest that net antioxidant capacity was not sufficiently strong to scavenge free radical activity. Resultantly OS was raised in RSD. These observations support our earlier findings.

The term OS denotes to imbalance between the production of reactive species and antioxidant defenses with a mild tilt in favor of oxidants. This results in a mild oxidizing environment which drives numerous reactions. This "mild OS" is an essential phenomenon of aerobic life. It regulates the processes like proliferation, migration, hypertrophy, differentiation, apoptosis, cytoskletal dynamics and metabolism. Redo state refers to the tendency or ability of cell or tissue to accept (more oxidized) or donate (more reduced) electrons. The OS is net result.

Raised OS has been incriminated in over hundred diseases including RSD. The proposed mechanism in RSD is that oxalate or crystals of oxalate or calcium phosphate in endothelium increase OS. The simmering OS causes renal endothelial cell injury, inflammation and death. The cell debris so obtained provides site for birth and growth of stone. In all the series we have examined so far, OS is consistently raised. Therefore, present study provides four evidences in favor of above hypothesis,: first, OS in erythrocytes was raised in RSD patients, second the blood and as well as urinary excretion of TBARs was higher, third citrate excretion was lower even in normo-oxalunic RSD patients and fourth both blood and urinary TBARS levels were still higher in hyper-oxaluric patients than normo-oxaluric patients

XO is a component of xanthine oxido-reductase, which has a principal role in xanthine metabolism. Xanthine-oxido-reductase is a house keeping enzymes which consists of two components viz XD (xanthing dehydrogenase) and XO. XD is primary gene product and XO is formed form XD through post translational modification. XD is primarily involved in uric acid synthesis with the help of NAD whereas XO produces predominantly numerous free radicals with the help of oxygen. It increases in injury and inflammation, thereby raising free radical pool. Thus it acts in kidney tissue as promoter of calcium oxalate nephrolithiasis, which is accompanied with both injury and inflammation. Based on this premise we measured XO activity in erythrocytes. It was significantly raised in RSD patients and therefore possibly involved in nephrolithiasis by raising OS. To best of our knowledge, this is being reported for the first time.

In the last exercise, 24 urinary excretions of calcium, oxalate, citrate and TBARS was measured in 42 males NS and 42 male RSD patients. Twenty Six RSD patients were normo-oxaluric (<40mg/24hrs) and 16 were hyperoxaluric (>40mg/24hrs). Simultaneously, erythrocyte TBAR level was also measured in these patients to assess OS. Compared to NS, even normo-oxaluric RSD patients had higher oxalate excretion. Hyper-oxaluric patients had still higher exertion of oxalate. Interestingly calcium, oxalate and TBARS exertion was raised in patients, while citric level was low. These observations suggest, that calcium excretion was consequent to increased oxalate exertion; and that raised TBARS was due to increased oxalate excretion. Hypo-citraturia is a common feature in stone formers in local population Strikingly XO activity was also raised in RSD patients. Notably, OS is raised in numerous diseases but is not accompanied by hyperoxaluria. On the contrary raised oxalate exertion is accompanied with raised OS. These observations intuitively suggest more oxidized environment (i.e. disturbed redox homeostasis) in patients.

Approximately, two third of renal calculi contain oxalate as major constituent. The percentage of oxalate in stones is still higher in this region (92.6%). Further, hyper-oxaluria and hypocitraturia are also very common here. Hypocitraturia weakens antioxidant defences thereby provoking OS. It is therefore logical to deduce that lop-sided redox is a risk factor in nephrolithiasis.

Admittedly mechanism of involvement of ROS and oxalate in the genesis of nephrolithiasis is still an overarching issue. However, the gathering evidence provides strong inkling of the association of hyperoxluria, hypocitraturia and elevated OS in the genesis of renal stones. A group of researchers from the University Of Iowa (USA) postulated that oxidative insult causes urothelial injury, which makes it more propitious to calcium oxalate deposition of which the end result is stone formation. Recently Khan[4] incisively examined this problem in detail. He postulated that renal epithelial exposure to high oxalate and crystals of calcium oxalate generate excess ROS, causing injury and inflammation in renal tubules. ROS and oxalate together in turn may cause cell death providing seed for crystal nucleation, making apical plasma membrane more adhesive to crystals or altering the availability of crystallization inhibiting proteins. Such changes would presumably promote nephrolithiasis by giving crystals times to grow, so they would be large enough to aggregate or attach to a growing stone on Randall plaques upon reaching the pelvis.

As a corollary, our data taken collectively indicate that antioxidant defenses are slightly inadequate and should be optimized by dietary improvements or therapeutic supplements, that oxalate in vivo increases OS; that oxalate in collusion with askew OS, is likely to increase the risk of renal stone; and that hypocitraturia exacerbates this risk.

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