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ORIGINAL ARTICLE

Circadian Periodicity of Circulating Plasma Lipid Peroxides, Uric Acid and Ascorbic Acid in Renal Stone Formers

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Abstract Circadian periodicity of plasma lipid peroxides and serum ascorbic acid and uric acid levels were studied in one hundred renal stone formers (55 women and 45 men; age 20–60 years) and 50 clinically healthy volunteers (21 women and 29 men; age 21–45 years) with diurnal activity from 06:00 to 22:00 and nocturnal rest. A marked circadian variation was demonstrated by population-mean-cosinor for all studied variables in stone formers and healthy subjects. By comparison to the healthy controls, parameter tests indicate that the stone formers had a higher MESOR (±SE) of MDA (2.90 ± 0.03 vs. 2.28 ± 0.06; F = 94.929, $p < 0.001$), a lower MESOR of serum ascorbic acid (0.722 ± 0.010 vs. 0.839 ± 0.10; F = 32.083, $p < 0.001$), and a similar MESOR of serum uric acid. Furthermore, the patients also differed from the healthy subjects in terms of their circadian amplitude and acrophase (tested jointly) of all three variables ($p < 0.001$). The demonstration herein of a circadian rhythm in MDA, serum ascorbic and uric acid suggests that these variables could also serve as markers to optimize the timing of treatment and to assess the patient's response to treatment for further management.

Keywords Circadian periodicity · Lipid peroxidation · MDA · Ascorbic acid · Uric acid · Renal stone formers

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Introduction

Urolithiasis is calculus formation at any level in the urinary collecting system, but most often the calculi arise in the kidney [1]. Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors [2]. The overall probability of forming stones differs in various parts of the world and is estimated as 1–5 % in Asia, 5–9 % in Europe, and 13 % in North America [3]; the recurrence rate of renal stones is about 75 % within 20 years [4]. In NHANES III (1988–1994) report, the prevalence of kidney stones was 5.25 %; it was higher in males, white/non-Hispanics, diabetics and those with hypertension [5]. Kidney stones are poly crystalline aggregations composed of varying amounts of crystalloid and organic matrix [6].

Many biological functions follow a circadian rhythm driven by internal and external cues that synchronize and coordinate organ physiology to changes along the 24-h scale in the environment and behavior. In renal stone formers, 6 variables (calcium, oxalic acid, and glycolic acid in particular) had no detectable circadian rhythm. However, an ultradian rhythm was demonstrated for calcium and oxalic acid with peaks being located around 02:00, 16:00 and 18:00. The risk of calcium-oxalate crystallization thus appears to be greater at these hours [7]. The biological rhythmicity and crystallization during the formation of calcium oxalate stone, studied by scanning electron microscope (SEM), has been attributed in part to the circadian or circannual rhythm in urinary excretion [8].

A few facts seem to indicate a possible pathogenetic relationship between hyperuricosuria and calcium oxalate stone formation. Hyperuricosuria has been reported to occur in up to 32 % of patients with calcium oxalate stones [9]. The frequency of calcium oxalate stone formation among patients with gout is high [10, 11]. Under physiological conditions, mammalian cells produce a small amount of reactive oxygen species (ROS) which influence biological processes. At high concentrations, ROS are the mediator of damage to lipids, proteins and nucleic acids. Exposure to free radicals has led organisms to develop a series of defense mechanisms that can protect against oxidative stress. The defense mechanisms are represented by non-enzymatic antioxidants (ascorbic acid, uric acid, vitamin E, carotenoids) and enzymatic antioxidants that are mainly represented by superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GPx), and glutathione reductase (GR) [12]. Oxidative stress has been implicated in the development of several disease processes, including diabetes, hypertension, cardiovascular disease, and obesity. The formation of kidney stones has also been linked to high oxidative stress and damage to kidney cells in humans. Reportedly, ascorbic acid and SOD were statistically significantly lower, whereas CAT activity was statistically significantly higher, as were thiobarbituric acid reactive substances (TBARS) in stone formers by comparison to their corresponding healthy counterparts [13].

Urinary excretion and urinary urate concentration are circadian periodic in stone formers with the highest excretion during the day time [14]. Urinary urate also exhibits a circadian rhythm in healthy subjects and in stone formers, reaching a maximum between 12:00 and 18:00 and a minimum between 00:00 and 06:00 [15]. A similar rhythm has also been reported by Lang et al. [16] and by Schneeberger et al. [17], although with different peak times.

Clinical studies have provided evidence for the development of oxidative stress in the kidneys of stone forming patients. Renal disorders which lead to oxidative stress appear to be a continuum. Stress produced by one disorder may trigger the other under the right circumstances. Chronomics of circulating plasma lipid peroxides, antioxidant enzymes and other small molecules have been reported in different pathological conditions [18–21]. Similarly, chronomics of circulating plasma lipid components and the effect of gender, age, diet and smoking status has recently been reported in healthy Indians of different age groups [22, 23]. However, there are few reports regarding the circadian nature of lipid peroxides, uric acid and ascorbic acid in renal stone formers. The present study aims to fill this gap by providing estimates of circadian characteristics of malondialdehyde (MDA), ascorbic acid and uric acid in stone formers and in clinically healthy controls in an attempt to understand the mechanism of oxidative stress in the etiopathogenesis of urolithiasis.

Materials and Methods

This study included one hundred renal stone formers (55 women and 45 men), 20–60 years of age, and 50 clinically healthy volunteers (21 women and 29 men), 21–45 years of age, consisting mainly of medical students, staff members and their families. Renal stone formers of both genders were admitted at the surgical wards of the Shri Mahant Indiresh Hospital, Shri Guru Ram Rai Institute of Medical and Health Sciences, Patel Nagar, Dehradun. They were synchronized for 1 week to a schedule of diurnal activity from 06:00 to 22:00 and nocturnal rest. All subjects took their usual (although not identical) meals three times daily (breakfast around 08:30, lunch around 13:00, and dinner around 20:30) with usual fluid intake which was neither altered nor recorded. They were not taking any drug/nutraceutical which could alter lipid peroxides or antioxidant status.

The study was approved by Institutional Ethics Committee. After signing a written informed consent, 6 ml of blood was collected from each study participant in plain and sterile vials containing heparin as anticoagulant at 6-hourly intervals over 24 h (at 06:00 12:00, 18:00 and 00:00). MDA [24], ascorbic acid [25] and uric acid [26] were determined spectrophotometrically.

Data of all participants were evaluated by conventional statistical analyses and by single and population-mean cosinor [27–29] for determination of the MESOR (Midline Estimating Statistic of Rhythm, a rhythm-adjusted mean), the circadian double amplitude (a measure of the extent of predictable change within a day), and the circadian acrophase (a measure of the timing of overall high values recurring each day). Parameter tests were performed to test the equality of the MESOR, 24-h amplitude and acrophase (considered singly or jointly) of each variable between the renal stone formers and the healthy volunteers. A p value <0.05 was considered to indicate statistical significance.

Results

Results are summarized in Tables 1 and 2. A marked circadian variation was demonstrated by population-mean-cosinor for all variables investigated herein in healthy volunteers and in stone formers ($p < 0.001$), Table 1. By comparison to the healthy controls, parameter tests indicate that the stone former patients had a higher MESOR (±SE) of MDA (2.90 ± 0.03 vs. 2.28 ± 0.06; $F = 94.929$, $p < 0.001$), a lower MESOR of serum ascorbic acid (0.722 ± 0.010 vs. 0.839 ± 0.10; $F = 32.083$, $p < 0.001$),

Table 1 Circadian variation of plasma MDA, ascorbic acid, and uric acid in renal stone formers and age-matched healthy controls

Variable	Clinical health (k = 50)					Stone formers (k = 100)				
	PR (%)	*p* value	M ± SE	2A (95 % CI)	ϕ (95 % CI)	PR (%)	*p* value	M ± SE	2A (95 % CI)	ϕ (95 % CI)
MDA (nmol/ml)	77	<0.001	2.28 ± 0.06	0.90 (0.70, 1.12)	−253 (−237, −267)	78	<0.001	2.90 ± 0.03	0.72 (0.60, 0.84)	−207 (−197, −218)
Ascorbic acid (mg/dl)	88	<0.001	0.839 ± 0.021	0.448 (0.376, 0.518)	−243 (−233, −254)	88	<0.001	0.722 ± 0.010	0.266 (0.238, 0.296)	−228 (−222, −235)
Uric acid (mg/dl)	73	<0.001	5.17 ± 0.08	1.06 (0.80, 1.32)	−131 (−115, −147)	78	<0.001	5.17 ± 0.06	0.86 (0.72, 1.00)	−190 (−178, −201)

MDA, Malondialdehyde; PR, percent rhythm, average proportion of variance accounted for by fit of 24-h cosine curve to individual data series; P, *p* value from zero-amplitude (no-rhythm) test; M, MESOR, a rhythm-adjusted mean value; 2A, double circadian amplitude, a measure of extent of predictable change within a day; ϕ, acrophase, measure of the timing of overall high values recurring each day, expressed in (negative) degrees with 360° ≡ 24 h and 0° = 00:00, SE, standard error; CI, confidence interval

and a similar MESOR of serum uric acid (Table 2). The patients also differed from the healthy subjects in terms of their circadian amplitude and acrophase (tested jointly) of all three variables ($p < 0.001$), Table 2. Stone formers had a smaller circadian amplitude than healthy controls (MDA: $F = 4.748$, $p = 0.031$; ascorbic acid: $F = 29.438$, $p < 0.001$; uric acid: $F = 4.598$, $p = 0.034$), Table 2. They had an earlier circadian acrophase of MDA (13:48 vs. 16:52) and ascorbic acid (15:12 vs. 16:12), but a later circadian acrophase of uric acid (12:40 vs. 08:44), Table 2.

Discussion

In our study, we recorded a marked circadian variation in MDA, ascorbic acid and uric acid concentrations in healthy Indians and renal stone formers. The lipid peroxidation and antioxidant enzymes play a role in the pathogenesis of renal stone. Various studies found the peroxidative stress to be higher in stone formers of all age groups as compared to their healthy counterparts [13, 30]. We confirmed that plasma lipid peroxides were elevated in stone formers. Moreover, we found that stone formers had an advanced circadian acrophase, which occurred about 3 h earlier than in our healthy controls, a result not previously reported to our knowledge. The role of lipid peroxidation and oxidative function in the pathogenesis of urolithiasis has been previously described [30]. Lipid peroxidation in cell membranes and subcellular organelles has been proposed as a primary mechanism for cellular membrane dysfunction and tissue injury associated with free-radical initiated processes. Renal stone disease is associated with high oxidative stress and damage to renal tubular cells [31, 32]. Oxidative stress is one of the major contributors for the development of stone formation. The increase in MDA concentrations observed herein could be due to increased oxidative stress in the kidneys from various sources or to a decrease in antioxidant defense mechanisms and vice versa.

Although much is known about the chemistry of lipid peroxidation and cellular defense mechanisms, chronobiological studies are needed to quantify the various cellular components involved in these processes to achieve better efficacy and safety of novel therapies for the management and prevention of recurrence of the disease. Circadian rhythms of pro-oxidants have been mapped to explore their putative chronotherapeutic role as markers in the prevention and management of different types of malignancies, pulmonary tuberculosis and cirrhosis of the liver [18–21]. Similarly, chronomics of circulating plasma lipid components in addition to effects of gender and age, diet and smoking were found to affect the MESOR of circulating plasma lipid components in healthy Indians. Age also

Table 2 Comparison of circadian rhythm parameters between renal stone formers and healthy volunteers

Variable	Population	MESOR	24 h Amplitude (A)	24 h acrophase (ϕ)	(A, ϕ)
MDA	Health	2.28	0.45	−253	
	Stone formers	2.90	0.36	−207	
	Comparison (F, P):	(94.929, <0.001)	(4.748, 0.031)	(23.501, <0.001)	(12.419, <0.001)
Ascorbic acid	Health	0.839	0.224	−243	
	Stone formers	0.722	0.133	−228	
	Comparison (F, P):	(32.083, <0.001)	(29.438, <0.001)	(6.285, 0.013)	(25.620, <0.001)
Uric acid	Health	5.17	0.53	−131	
	Stone formers	5.17	0.43	−190	
	Comparison (F, P):	(0.001, 0.972)	(4.598, 0.034)	(38.444, <0.001)	(17.981, <0.001)

MDA, Malondialdehyde; P, *p* value from F-test comparing MESOR, 24-h amplitude and acrophase, separately or jointly (A, ϕ) between the 100 stone formers and the 50 healthy controls; acrophase expressed in (negative) degrees with 360° ≡ 24 h and 0° = 00:00

affected the circadian amplitude of these variables indicating the possibility of using non-pharmacological interventions to improve a patient's metabolic profile before prescribing medication under near normal tropical conditions [22, 23].

No difference was found in the MESOR of serum uric acid between the stone formers and the healthy subjects. A marked circadian variation was recorded in both groups. In a previous study, it was reported that the uric acid excretory pattern was not statistically significantly different in stone formers from that in healthy controls, although the excretion was a little higher in the stone formers [15]. Maximal urate excretion around 11:21 (around midday) may correspond to the peak time for the crystallization of stones containing an admixture of urate in stone formers. If so, it could be of clinical significance when trying to inhibit crystallization of renal stones and to minimize the risk of crystal growth (urate admixture) in renal tubules [15].

Serum ascorbic acid also showed a statistically significant circadian variation in healthy volunteers, peaking around 16:12. The serum ascorbate concentration was found to be lower at all sampling times in stone formers, with a smaller circadian amplitude. Chalmeu et al. [33] reported a lower excretion rate of ascorbate in urolithiasis. Ascorbic acid may be endogenously converted to oxalate and increase the absorption of dietary oxalate, which in turn may induce free radical generation, thereby causing renal stones [34]. Hyperoxaluria found in urolithiasis patients induces calcium oxalate crystal deposition in the kidney. Oxalate crystals are the main component of renal stones [35]. Our results indicate a role of lipid peroxidation and oxidation function in the pathogenesis of urolithiasis, as observed from the negative correlation between MDA and other antioxidants. Modern lifestyle changes, sedentary habits, lack of easiness, an unhealthy dietary plan, and overweight problems of the affluent societies emerge to be important promoters of the "stone-boom" in the millennium, both in developed and undeveloped countries. Major risk factors that contribute to stone formation and its recurrence include "classic" risk factors in the urine, epidemiological factors (climate, race, ethnicity, age, gender, body weight) [2].The demonstration herein of a circadian rhythm in MDA, serum ascorbic and uric acid suggests that these variables could also serve as markers to optimize the timing of treatment administration and to assess the patient's response to treatment. Further studies are needed to correlate the lipid peroxide concentrations with free-radical scavengers, their nature, status and rhythm after administration of known dietary and therapeutic antioxidants thereby opening a new chapter in our understanding of the pathogenesis and management of renal stone disease.

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Compliance with Ethical Standards

Conflict of interest None.

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