Chronomics of Circulating Plasma Lipid Peroxides and Antioxidant Enzymes in Renal Stone Formers

Rajeev Singh Kushwaha^{1,2} · R. C. Gupta¹ · Sumita Sharma² · Tariq Masood² · J. P. Sharma³ · Rajesh K. Singh⁴ · Raj K. Singh⁴ · Cathy L. Gierke⁵ · Germaine Cornelissen⁵

Received: 25 September 2017 / Accepted: 8 December 2017 / Published online: 21 December 2017
© Association of Clinical Biochemists of India 2017

Abstract The chronome of lipid peroxidation and antioxidant defense mechanisms may relate to the efficacy and management of time qualified preventive therapeutic and dietary interventions. One hundred renal stone patients, 20–60 years of age, and 50 clinically healthy volunteers, 21–45 years, were synchronized for 1 week with diurnal activity from 06:00 to 22:00 and nocturnal rest. All subjects took their usual meals three times daily (breakfast around 08:30, lunch around 13:00, and dinner around 20:30) with usual fluid intake. Drugs known to affect free radical system were not taken. Blood samples were collected at 6-h intervals for 24-h under standardized, presumably 24-h synchronized conditions. Determinations included plasma lipid peroxides, in terms of malondialdehyde (MDA) and blood superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) activities. A marked circadian variation was demonstrated for each studied variable by population-mean cosinor in renal stone patients and healthy participants ($p < 0.001$). By comparison to healthy subjects,

parameter tests indicate that the stone formers had a higher MESOR of MDA, but a lower MESOR of SOD, GPx, GR and CAT. Furthermore, the patients also differed from the healthy controls in terms of their circadian amplitude and acrophase (tested jointly) of all variables ($p < 0.001$). Mapping the broader time structure with multifrequency circadian characteristics of oxidants and anti-oxidants is needed for exploring their role as marker in the treatment and management of urolithiasis.

Keywords Chronomics · Lipid peroxides · MDA · SOD · GPx · GR · CAT · Renal stone formers · Urolithiasis

Introduction

During the last few years, there has been significant progress in both understanding and management of urolithiasis. The importance of urine biochemistry was questioned by several investigators years ago, however, the decisive role of cellular process (induced by oxidative stress) and the renal papilla has only recently been generally accepted as the most important step in stone formation [1]. Urolithiasis is characterized by the formation and retention of solid crystals within the urinary tract. Kidney stones are mostly composed of calcium oxalate, which predominantly generates free radicals that are toxic to renal tubular cells [2]. For calcium oxalate urolithiasis, the formation of papillary calcifications plays a key role and is of prognostic relevance [1]. Urolithiasis places a significant economic burden on the healthcare system, especially in industrialized countries where, owing to changes in lifestyle and diet, the incidence of stone disease has steadily increased over the last several decades; unfortunately, it will probably continue to increase for a number of reasons, one of

✉ Raj K. Singh
singhrk23a@hotmail.com

¹ Department of Biochemistry, NIMS Medical College and Hospital, Shobha Nagar, Jaipur, Rajasthan 303121, India

² Department of Biochemistry, SGRR Institute of Medical and Health Sciences, Dehradun 248001, India

³ Department of Surgery, SGRR Institute of Medical and Health Sciences and Shri Mahant Indires Hospital, Dehradun, UK 248001, India

⁴ Department of Biochemistry, TS Misra Medical College and Hospital, Amausi, Lucknow, UP 226008, India

⁵ Halberg Chronobiology Center, University of Minnesota, Minneapolis, MN 55455, USA

which is global warming [3, 4]. This is common in idiopathic calcium oxalate (Ca–Ox) stone formers (ICSF), by which we mean Ca Ox stone formers with no known systemic disease apart from familial (idiopathic) hypercalciuria. Renal circadian rhythms are influenced by external cues such as feeding, ambient light, and activity, as well as inherently by intrinsic clocks [5, 6]. Urolithiasis has been a documented medical affliction since at least ancient Egyptian civilization [7] and continues to be responsible for an increasing number of practitioner visits worldwide [8, 9]. The circadian rhythmicity has been found to be altered in the calcium oxalate renal stone formers. There was loss of circadian rhythm of certain variables while an 8-h ultradian rhythm occurred in stone formers. Circadian periodicity of urinary inhibitor of calcium oxalate crystallization has been reported in normals and renal stone formers [10]. Similarly, circadian dietary antioxidant vitamin deficiency may also be a contributing factor in oxidative stress and stone formation [11].

Experimental and animal studies have shown crystal-induced oxidative stress and tubular injury in kidney cells [12, 13]. Hence, oxidative stress is a causal factor and key promotor of urolithiasis associated with renal tubular epithelium cell injury. Clinical studies have also 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 [14–17]. Similarly, circadian periodicity of plasma lipid peroxides and serum uric acid and ascorbic acid have been recently reported in renal stone formers [18]. However, there are few reports regarding the circadian nature of oxidants and antioxidant enzymes in stone formers. The present study aims to fill this gap by providing circadian profiles of circulating plasma lipid peroxides and antioxidant enzymes in renal stone formers in an attempt to understand the role of these variables in the pathogenesis of urolithiasis.

Materials and Methods

Patient Accrual

This study included one hundred clinically confirmed renal stone formers of either gender in the age range of 20–60 years and 50 clinically healthy volunteers, which consisted mainly of medical students, staff members and their families. The age of healthy volunteers ranged from 21 to 45 years. All the subjects were studied for circadian

dynamics in terms of the Midline Estimating Statistic of Rhythm (MESOR), Amplitude (half the predictable extent of change within a day) and Acrophase (timing of overall high values recurring each day) of different biochemical variables. This study was approved by the Institutional Ethical Committee of the SGRR Institute of Medical Sciences, Dehradun, India. Renal stone formers of both genders were admitted at the Surgical Wards of Shri Mahant Indresh Hospital, Shri Guru Ram Rai Institute of Medical & Health Sciences, Patel Nagar, Dehradun. They were synchronized for 1 week with 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 the status and/or rhythm of variables examined herein. After obtaining their written informed consent, 6 ml of blood was collected from each subject in plain and sterile vials containing heparin as anticoagulant at 6-h intervals, i.e., at 06:00 12:00, 18:00 and 00:00 h to cover a complete 24-h period. Plasma lipid peroxides in terms of malondialdehyde (MDA) [19] and antioxidant enzymes in terms of superoxide dismutase (SOD) [20], glutathione peroxidase (GPx) [21], glutathione reductase (GR) [22] and catalase (CAT) [23] were determined spectrophotometrically.

Statistical Analysis

Data of all participants (healthy volunteers and stone formers) were evaluated by conventional statistical analyses and by the single and population-mean cosinor procedures [24–26]. Accordingly, the MESOR (a rhythm-adjusted mean), the circadian amplitude and the circadian acrophase were determined. Population-mean cosinor parameters of the fitted 24-h component were compared between the healthy controls and the stone formers by parameter tests [25]. A *p* value less than 0.05 was considered to indicate statistical significance.

Results

Circadian rhythm characteristics 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 ($\pm$ SE) of MDA (2.90 ± 0.03 vs. 2.28 ± 0.07 ; $F = 94.929$, $p < 0.001$), but a lower MESOR of SOD (16.210 ± 0.09 vs. 19.89 ± 0.17 ;

Table 1 Circadian variation of plasma MDA concentration and SOD, GPx, GR, Catalase activity in renal stone formers and Age-matched healthy controls

Variable	Clinical health (n = 50)					Stone formers (n = 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/m)	77	< 0.001	2.28 ± 0.07	0.91 (0.70, 1.11)	– 253° (– 237, – 267)	78	<0.001	2.90 ± 0.03	0.72 (0.60, 0.84)	– 207° (– 197, – 218)
SOD (Units/ml RBCs)	83	< 0.001	19.89 ± 0.17	2.67 (2.10, 3.24)	– 110° (– 99, – 123)	78	<0.001	16.21 ± 0.09	1.79 (1.51, 2.06)	– 118° (– 110, – 125)
GPx (nmole NADH Oxidative/min/mg/Protein)	85	< 0.001	4.05 ± 0.03	0.74 (0.52, 0.96)	– 201° (– 172, – 227)	78	<0.001	3.16 ± 0.04	0.76 (0.66, 0.87)	– 228° (– 218, – 237)
GR (nmole NADH Oxid/min/mg/Protein)	87	< 0.001	5.02 ± 0.03	1.49 (1.32, 1.65)	– 125° (– 119, – 131)	77	<0.001	3.52 ± 0.08	0.81 (0.71, 0.92)	– 144° (– 136, – 152)
Catalase (units/ml RBCs)	82	< 0.001	14.46 ± 0.07	2.09 (1.84, 2.34)	– 120° (– 112, – 127)	77	<0.001	13.66 ± 0.11	1.44 (1.22, 1.65)	– 133° (– 126, – 141)

MDA Malondialdehyde; SOD Superoxide dismutase; GPx Glutathione peroxidase; GR Glutathione reductase; CAT Catalase, n number of subjects, PR percentage rhythm, average proportion of variance accounted for by fit of 24-h cosine curve to individual data series; p p value from zero amplitude (norhythm) test; M: MESOR, a rhythm-adjusted mean value; 2A Double Amplitude, extent of predictable change within a day; φ Acrophase, measure of the timing of overall high recurring each day, expressed in (negative) degrees, with 360° = 24 h and 0° = 00:00; CI confidence interval

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

Variable	Population	MESOR	24-h Amplitude (A)	24-h Acrophase (ϕ)	(A, ϕ)
MDA	Healthy controls	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)
SOD	Healthy controls	19.89	1.34	– 110	
	Stone formers	16.21	0.89	– 118	
	Comparison (F, P)	(422.427, < 0.001)	(10.035, 0.002)	(1.305, NS)	(5.320, 0.0054)
GPx	Healthy controls	4.05	0.37	– 201	
	Stone formers	3.16	0.38	– 228	
	Comparison (F, P)	(206.988, < 0.001)	(0.153, NS)	(5.159, 0.025)	(2.629, 0.074)
GR	Healthy controls	5.02	0.74	– 125	
	Stone formers	3.52	0.41	– 144	
	Comparison (F, P)	(150.573, < 0.001)	(52.007, < 0.001)	(14.836, < 0.001)	(27.251, < 0.001)
Catalase	Healthy controls	14.46	1.05	– 120	
	Stone formers	13.66	0.72	– 133	
	Comparison (F, P)	(22.964, < 0.001)	(13.568, < 0.001)	(6.708, 0.011)	(10.298, < 0.001)

MDA Malondialdehyde; SOD Superoxide dismutase; GPx Glutathione peroxidase; GR Glutathione reductase; CAT Catalase; P *p* value from F-test testing the equality of parameter(s): MESOR, 24-h amplitude and acrophase, separately or jointly (A, ϕ) between the 100 stone formers and 50 healthy controls; acrophase expressed in (negative) degrees with $360^\circ = 24$ h and $0^\circ = 00:00$; NS not significant ($p > 0.10$)

$F = 422.427$, $p < 0.001$). Stone formers also had a lower MESOR of GR (3.52 ± 0.08 vs. 5.02 ± 0.03 ; $F = 150.573$, $p < 0.001$), a lower MESOR of GPx (3.16 ± 0.034 vs. 4.05 ± 0.03 ; $F = 206.988$, $p < 0.001$), and a lower MESOR of CAT (13.66 ± 0.011 vs. 14.46 ± 0.07 ; $F = 22.964$, $p < 0.001$). The patients also differed from the healthy subjects in terms of their circadian amplitude and acrophase (tested jointly) of all variables ($p < 0.001$), except for GPx ($p = 0.074$), Table 2. As compared to healthy controls, stone formers had a smaller circadian amplitude of MDA ($F = 4.748$, $p = 0.031$), SOD ($F = 10.035$, $p = 0.002$), GR ($F = 52.007$, $p < 0.001$), and CAT ($F = 13.568$, $p < 0.001$). The circadian amplitude of GPx did not differ significantly between the two groups. As compared to healthy controls, stone formers had an earlier circadian acrophase of MDA (13:48 vs. 16:52), but a later circadian acrophase of GPx, GR, and CAT activity. The circadian acrophase of SOD activity did not differ between the two groups (Tables 1 and 2).

Discussion

A marked circadian variation in MDA concentration and in the activity of SOD, GPx, GR and CAT was recorded in healthy Indians and renal stone formers in the present study. Moreover, we found that stone formers had an advanced circadian acrophase of MDA, which occurred about 3 h earlier than in healthy controls. Renal stone

disease has been reported to be associated with high oxidative stress and damage to renal tubular cells [8, 27]. Oxidative stress is one of the major contributors in stone formation. It has also been reported that plasma lipid peroxide concentrations reflected the severity of a disease process and it also indicated the occurrence of membrane damage in cell brought on by the disease [28]. 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.

The biological clock is involved in many aspects of our complex physiology. We now know that all multicellular organisms, including humans, utilize a similar mechanism to coordinate circadian rhythms. Beyond three great Nobel Laureates in Physiology or Medicine for 2017, Prof. Jeffrey C Hall, Prof. Michael Rosbash and Prof. Michael W Young for their elucidation of the molecular mechanisms underlying circadian rhythms, Prof. Franz Halberg, the father of chronobiology and chronomedicine was the first to demonstrate that all biological functions, including eosinophil counts, blood pressure and heart rate, vary predictably according to a broad time structure, circadian rhythms in particular [29, 30].

In a biological system, many biological functions follow circadian rhythms driven by internal and external cues that synchronize and coordinate organ physiology to 24-h changes in the environment and behavior. In mammalian biology, many aspects of behavior and physiology follow

circadian rhythms which are generally presumed to confer adaptive advantage by coordinating behavior and organ physiology to ambient day-and-night cycles [12]. In the kidney, significant circadian rhythms exist for multiple renal hemodynamic, glomerular and tubular parameters [31, 32]. The circadian rhythmicity has been found to be altered in the calcium oxalate renal stone formers. There was loss of circadian rhythm of certain variables while an 8-h ultradian rhythm occurred in stone formers. The biological rhythmicity and crystallization during the formation of calcium oxalate stone, studied by Scanning Electron Microscope (SEM), has been attributed to the 24-h or annual rhythm in urinary excretion [28].

Superoxide dismutases are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide [33, 34]. There were marked differences in SOD activity among healthy volunteers at different sampling times, but the acrophase was similar for healthy controls (recorded at 07:02) and stone formers (recorded at 07:52). SOD activity was found to be lower at all sampling times in patients who had a significantly lower MESOR. To our knowledge, such results have not been reported earlier. CAT activity was also found to be lower at all sampling times in stone formers, who also had a smaller circadian amplitude on a population basis, and a lower MESOR. However, GPx activity was found to be lower in stone formers who had a circadian amplitude similar to that of the healthy controls but a circadian acrophase occurring almost 2 h later, around 15:12.

A significant circadian variation was also recorded for GR activity in stone formers with maximal and minimal activity at 06:00 and 00:00, respectively. Both the MESOR and circadian amplitude were decreased in stone formers as compared to the healthy controls. Moreover, the activity was found to be decreased at all studied time points like other antioxidant enzymes. Decreased SOD, CAT, GPx and GR activities in stone formers might cause accumulation of ROS due to inter-dependence of these enzymes. The circadian pattern of these enzymes was observed to be changed in kidney stone formers with decreased activity at all time points tested along the 24-h cycle as compared to that of healthy controls. The circadian pattern of GPx activity was found to be shifted in patients who had a circadian acrophase peaking almost 2 h later than in health. Such an alteration in stone formers shows the importance of these antioxidant enzymes, which may serve as an important marker for the early diagnosis and management of the disease.

Stone formers had reduced activities of antioxidant enzymes SOD, CAT, GPx and GR, indicating that the system of defense against free radicals is severely impaired in stone formers. In a recent study in patients of

kidney and ureteral stones, the authors reported that the erythrocytes of stone groups had significantly lower concentrations and activities of antioxidant proteins, namely, reduced glutathione, catalase, and copper- or zinc-superoxide dismutase as compared to the erythrocytes of controls. The ureteral stone group also had significantly lower erythrocyte glutathione peroxidase concentrations and glutathione reductase activity than the controls. Erythrocyte oxalate concentrations correlated positively with erythrocyte MDA concentrations and negatively with erythrocyte antioxidant protein activities. Erythrocyte oxidative stress, as indicated by cellular MDA concentrations, also correlated well with urinary glutathione S-transferases and stone size [35].

Low concentrations of antioxidant enzymes not only favor free radical accumulation, but it is also possible that the scavenger system itself is impaired as a consequence of reduced synthesis of these enzymes in stone formers [34]. A previous research has also shown greater urinary MDA, plasma MDA, and urinary NAG activity but diminished glutathione (GSH), cellular glutathione peroxidase (cGPx), protein thiol, and vitamin E activity in patients diagnosed with kidney stones. After supplementation with potassium citrate (60 mEq/day for 1 month), the patients showed decreased urinary MDA, plasma MDA, and increased vitamin E [36].

To date, there are only a few reports regarding the circadian nature of lipid peroxides and antioxidant enzymes (SOD, CAT, GPx, GR) in renal stone formers living under near tropical conditions. With the global warming debate, much attention has been given to the changes in human health caused by increased temperature, as well as to the increase in the incidence of nephrolithiasis related to climate change [37, 38]. Dietary changes have led to an increase in body weight, which has also been related to the increased risk of stone formation [39]. The demonstration herein of a circadian rhythm in serum MDA, SOD, CAT, GPx, and GR suggests that these variables could 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.

Acknowledgements The authors are grateful to Hon. Chairman, Shri Guru Ram Rai Education Mission for his constant support and guidance in pursuing such studies in our laboratory. We are also indebted to the staff of the Department of Biochemistry, SGRR Institute of Medical and Health Sciences for their technical assistance. Our special thanks to Dr Rachit Garg for patient enrolment.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Strohmaier WL. Recent advances in understanding and managing urolithiasis [version1; referees:3 approved]. F1000Research. 2016;5(F1000 Rev):2651.
2. Kizivat T, Smolic M, Maric I, Levak ML, Smolic R, Curcic IB, et al. Antioxidant pre-treatment reduces the toxic effects of oxalate on renal epithelial cells in a cell culture model of urolithiasis. *Int J Environ Res Public Health*. 2017;14:109–33.
3. Hesse A, Brändle E, Wilbert D, Köhrmann KU, Alken P. Study on the prevalence and incidence of urolithiasis in Germany comparing the years 1979 versus 2000. *Eur Urol*. 2003;44(6):709–13.
4. Strohmaier WL. Economics of stone disease/treatment. *Arab J Urol*. 2012;10(3):273–8.
5. Moore-Ede MC. Physiology of the circadian timing system: predictive versus reactive homeostasis. *Am J Physiol*. 1986;250:R737–52.
6. Moore-Ede MC, Herd JA. Renal electrolyte circadian rhythms: independence from feeding and activity patterns. *Am J Physiol*. 1977;232:F128–35.
7. Eknoyan G. History of urolithiasis. *Clin Rev Bone Miner Metabol*. 2004;2:177–85.
8. Ramello A, Vitale C, Marangella M. Epidemiology of nephrolithiasis. *J Nephrol*. 2000;13(Suppl):S45–50.
9. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976–1994. *Kidney Int*. 2003;63:1817–23.
10. Singh RK, Bansal A, Bansal SK, Singh AK, Mahdi AA. Circadian periodicity of urinary inhibitor of calcium oxalate crystallization in healthy Indians and renal stone formers. *Eur Urol*. 1993;24(3):387–92.
11. Singh RB, Niaz MA, Cornelissen G, Otsuka K, Siegelova J, Fiser B, et al. Circadian rhythmicity of circulating vitamin concentrations. *Scripta Medica (Brno)*. 2001;74(2):93–6.
12. Mills JN. Human circadian rhythms. *Physiol Rev*. 1966;46:128–71.
13. Berg W, Brundigg P, Bothor C, Schneider J. Biological rhythmicity and crystallization—urine profiles and Se-studies on calcium oxalate stone genesis. *Int Urol Nephrol*. 1982;14:363–72.
14. Singh R, Singh RK, Tripathi AK, Gupta N, Kumar A, Singh AK, et al. Circadian periodicity of plasma lipid peroxides and antioxidant enzymes in pulmonary tuberculosis. *Ind J Clin Biochem*. 2004;19(1):14–20.
15. Singh R, Singh RK, Mahdi AA, Singh RK, Kumar A, Tripathi AK, et al. Circadian periodicity of plasma lipid peroxides and other antioxidants as putative markers in gynecological malignancies. *In Vivo*. 2003;17:593–600.
16. Singh R, Singh RK, Tripathi AK, Cornelissen G, Schartzkopff O, Otsuka K, et al. Chronomics of circulating plasma lipid peroxides, antioxidant enzymes and other related molecules in cirrhosis of liver. In the memory of late Shri Chetan Singh. *Biomed Pharmacother*. 2005;59(Suppl 1):S229–35.
17. Singh R, Singh RK, Masood T, Tripathi AK, Mahdi AA, Singh RK, et al. Circadian time structure of circulating plasma oxides, antioxidant enzymes and other small molecules in peptic ulcers. *Clin Chim Acta*. 2015;451:222–6.
18. Kushwaha RS, Gupta RC, Sharma JP, Sharma S, Singh RK, Cornelissen G. Circadian periodicity of plasma lipid peroxides, uric acid and ascorbic acid in renal stone formers. *Ind J Clin Biochem*. 2017;32(2):220–4.
19. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351–8.
20. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem*. 1969;244(22):6049–55.
21. Pagila DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967;2:158–69.
22. Hazelton GA, Lang CA. GSH content of tissue in aging mouse. *Biochem J*. 1980;188:25–30.
23. Aebi H, Suter H. Protective function of reduced glutathione against the effect of pro-oxidative substances and of irradiation in the red Cell. In: Flohe I, Benhar HC, Sies H, Waller HD, Wendel A, editors. *Glutathione*. Stuttgart: Georg Thieme; 1974. p. 192–9.
24. Halberg F, Johnson EA, Nelson W, Runge W, Sothorn R. Autorhythmometry-procedures for physiologic self-measurements and their analysis. *Physiol Teach*. 1972;1:1–11.
25. Bingham C, Arbogast B, Cornelissen G, Lee JK, Halberg F. Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*. 1982;9:397–439.
26. Cornelissen G, Halberg F. Chronomedicine. In: Armitage P, Colton T, editors. *Encyclopedia of biostatistics*, vol. 1. Chichester: Wiley; 1998. p. 642–9.
27. Elliot JS, Eusebio E. Calcium oxalate solubility. The effects of trace metals. *J Invest Urol*. 1967;9:428.
28. Tungsanga K, Sriboonlue P, Futrakul P, Yachantha C. Renal tubular cell damage and oxidative stress in renal stone patients and the effect of potassium citrate treatment. *Urol Res*. 2005;33(1):65–9.
29. Cornelissen G, Halberg E, Halberg F, Halberg J, Sampson M, Hillman D, Nelson W, Sánchez de la Peña S, Wu J, Delmore P, Marques N, Marques MD, Fernandez JR, Hermida RC, Guillaume F, Carandente F. Chronobiology: a frontier in biology and medicine. *Chronobiologia*. 1989;16:383–408.
30. Singh RB, Hristova K, Pella D, Feckado J, Chaves H, Mondal RN, et al. Extended consensus on guidelines for assessment of risk and management of hypertension. A scientific statement of the International College of Cardiology—Thank you Dr Franz Halberg. *World Heart J*. 2014;6(1):63–72.
31. Bonny O, Firsov D. Circadian clock and the concept of homeostasis. *Cell Cycle*. 2009;8:4015–6.
32. Firsov D, Bonny O. Circadian regulation of renal function. *Kidney Int*. 2010;78:640–5.
33. Zelko I, Mariani T, Folz R. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution and expression. *Free Radic Biol Med*. 2002;33:337–49.
34. Bannister J, Bannister W, Rotilio G. Aspects of the structure, function, and applications of superoxide dismutase. *CRC Crit Rev Biochem*. 1987;22:111–80.
35. Ma MC, Chen YS, Huang HS. Erythrocyte oxidative stress in patients with calcium oxalate stones correlates with stone size and renal tubular damage. *Urology*. 2014;83(2):510.
36. Khan SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. *Urol Res*. 2005;33(5):349–57.
37. Wilson JF. Facing an uncertain climate. *Ann Intern Med*. 2007;146:153–6.
38. Fakheri RJ, Goldfarb DS. Ambient temperature as a contributor to kidney stone formation: implications of global warming. *Kidney Int*. 2011;79:1178–85.
39. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. *JAMA*. 2005;293:455–62.