

Evaluating chronomic profiles of oxidative stress markers: Vegetarian versus non-vegetarian renal stone formers.

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Abstract

The effect of diet on the circadian nature of lipid peroxides and antioxidant enzymes may relate to the prevention and management of time-qualified protective interventions. This study includes 100 renal stone formers (age: 20-60 years; 50 vegetarians and 50 non-vegetarians) synchronised for one week to a diurnal activity period from 06:00 to 22:00 and nocturnal rest. Fifty healthy individuals (age: 21-45 years; 20 vegetarians and 30 non-vegetarians) were also included. All subjects took their 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 systems were not administered. Blood samples from each participant were collected at 6-hour intervals for 24 hours to determine the levels of MDA, SOD, CAT, GPx and GR. Data were analysed using the single-and population-mean cosinor to estimate the circadian rhythm characteristics. A marked circadian variation for all the studied variables was demonstrated by the population-mean cosinor ($p < 0.001$) and one-way analysis of variance ($p < 0.001$) in vegetarian and non-vegetarian controls and vegetarian and non-vegetarian stone formers. Compared to the healthy controls, the stone formers had high MDA ($p < 0.001$), low circadian amplitude ($p < 0.001$), and the circadian acrophase occurred earlier ($p < 0.001$). The demonstration of a circadian rhythm in all the studied variables suggests that these variables could also serve as putative markers to optimize the timing of drug administration and to assess the patients' response to the treatment.

Keywords: Diet, Vegetarian, Non-vegetarian, MDA, SOD, CAT, GPx, GR, Renal stone formers, Urolithiasis.

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Introduction

Kidney stones include stones of calcium, oxalate, uric acid, cystine, and xanthine [1]. Calcium oxalate stones are common. The overall probability of stone formation is estimated to be 1%-5% in Asia [2]. Reduced glutathione and antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and catalase, and some dietary antioxidants, such as tocopherol and ascorbic acid, play a key role in protecting cells against oxidative damage [3]. They may have a bearing on stone formation also. Kidney stone formation has been linked to high oxidative stress and damage to kidney cells in humans. In stone formers, ascorbic acid and superoxide dismutase were found to be lower, whereas catalase and the TBARS (Thiobarbituric Acid Reactive Substances) were higher [4,5].

There is circadian variation in the excretion and concentration of urinary urate in stone formers [6], with the highest excretion during the daytime. Urinary urate also exhibits a definite circadian rhythm in normal subjects and stone formers, with maximum levels from 1200 to 1800 hours and minimum levels from 0000 to 0600 hours, showing a definite rhythm [7]. Singh et al. and Chaudhari et al. [8-10] showed clear diurnal rhythmicity and significant levels in the plasma levels of

Malondialdehyde (MDA, a measure of lipid oxidation) and activity of the antioxidant enzymes Superoxide Dismutase (SOD), catalase, Glutathione Peroxidase (GPx) and Glutathione Reductase (GR) in healthy men and women. Many clinical studies have also provided evidence for the development of oxidative stress in the kidneys of stone-forming patients. Stress produced by one disorder may trigger another under the right circumstances. The chronomics of circulating plasma lipid peroxides, antioxidant enzymes, and other small molecules have been reported in different pathological conditions and stone formers [11-14]. However, there are few reports on the circadian nature of oxidants and antioxidants in stone formers.

Vegetarian diets have multiple beneficial effects, although they can also contribute to nutrient deficiencies such as vitamin B₁₂, vitamin D, iron, calcium, zinc, and omega-3 fatty acids [15]. Many studies have reported that vegetarian diets are associated with a lower risk of gout and hyperuricemia [16]. In addition, dietary habits can affect urinary pH. Omnivores have lower urinary pH and higher net acid excretion compared to vegetarians [17], and this situation may cause uric acid precipitation with crystallisation in the urine. The circadian periodicity and the effects of gender, age, diet and smoking on circulating plasma lipid peroxides and antioxidant enzymes

have already been reported in healthy Indians of different age groups [18]. Whether the nature of diet (vegetarian/non-vegetarian) can affect the circadian rhythm of oxidants and antioxidants in stone formers is not known. The present study was undertaken to assess and compare the circadian profiles of oxidants and antioxidants in vegetarian and non-vegetarian stone formers in an attempt to understand their role in urolithogenesis.

Materials and Methods

This study was conducted on 100 renal stone former patients of both sexes in the age range of 20 to 60 years. Out of these, 50 were vegetarians, and 50 were non-vegetarians. Fifty healthy individuals aged 21 to 45 years were included as controls, of whom 20 were vegetarians and 30 were non-vegetarians. Written consent was obtained from all the participants. The study was approved by the Institutional Ethics Committee of SGRRIM&HS, Dehradun.

All the subjects were studied for circadian rhythm 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. All renal stone formers were admitted to the surgery wards of Shri Mahant Indresh Hospital (SMIH), Shri Guru Ram Rai Institute of Medical & Health Sciences, Dehradun. They were synchronised for one week with diurnal activity from 0600 to 2200 hours and nocturnal rest. All subjects took their usual (though not identical) meals three times a day-breakfast at 08:30 hours, lunch at 13:00 hours, and dinner at 20:30 hours, with usual fluid intake which was neither altered nor recorded. They were not taking any drug/nutraceutical that could alter the status and rhythm of the studied variables.

Six mL of venous blood was collected from each subject in heparinized vials at 6-hourly intervals, *i.e.*, at 06:00, 12:00, 18:00 and 00:00 hours. Plasma lipid peroxides in terms of

malondialdehyde [19] and antioxidant status in terms of superoxide dismutase [20], catalase, glutathione peroxidase [21], and glutathione reductase [22] were measured in serum spectrophotometrically.

Statistical analysis

The data of all participants were evaluated using conventional statistical analyses and single and population mean cosinor procedures. Accordingly, the MESOR (Midline Estimating Statistic of Rhythm), a circadian 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) were determined. A p-value of <0.05 was considered to be significant.

Results

A marked circadian variation for all the studied variables was demonstrated by population mean cosinor ($p < 0.001$) and one-way analysis of variance ($p < 0.001$) in vegetarian and non-vegetarian controls and vegetarian and non-vegetarian stone formers. In comparison to the healthy controls, the stone formers had a high MESOR of MDA ($p < 0.001$), low circadian amplitude ($p < 0.001$), and the circadian acrophase occurred earlier ($p < 0.001$).

Table 1 shows that compared to the healthy vegetarian controls, vegetarian stone patients had a higher MESOR ($\pm$ CI) of MDA (2.88 ± 0.10 vs. 2.23 ± 0.22 ; $F=57.31$, $p < 0.001$), but a lower MESOR of SOD (16.38 ± 0.31 vs. 19.88 ± 0.62 , $p < 0.001$). Stone formers also had a lower MESOR of catalase (13.89 ± 0.32 vs. 14.56 ± 0.18 , $p < 0.001$), a lower MESOR of GPx (3.18 ± 0.11 vs. 4.08 ± 0.10 , $p < 0.001$); and a lower MESOR of GR (3.43 ± 0.25 vs. 5.04 ± 0.10 , $p < 0.001$).

Table 1. Circadian rhythm of plasma MDA, SOD, catalase, GPx, and GR in vegetarian renal stone formers and vegetarian controls.

Variable	Healthy controls (k=30)				Vegetarian stone formers (k=50)			
	p-value	M $\pm$ CI	A $\pm$ CI	ϕ (95% CI)	p-value	M $\pm$ CI	A $\pm$ CI	ϕ (95% CI)
MDA (nmol/m)	<0.001	2.23 ± 0.22	0.41 ± 0.18	$-260^\circ (-235^\circ, -289^\circ)$	<0.001	2.88 ± 0.10	0.38 ± 0.08	$-207^\circ (192^\circ, -223^\circ)$
SOD (units/ml RBCs)	<0.001	19.88 ± 0.62	1.19 ± 0.49	$-113^\circ (-90^\circ, -134^\circ)$	<0.001	16.38 ± 0.31	1.01 ± 0.18	$-118^\circ (-107^\circ, -127^\circ)$
Catalase (units/ml RBCs)	<0.001	14.56 ± 0.18	1.21 ± 0.18	$-119^\circ (-108^\circ, -129^\circ)$	<0.001	13.89 ± 0.32	0.68 ± 0.15	$-137^\circ (-126^\circ, -148^\circ)$
GPx (nmol NADH Oxi/min/mg/protein)	<0.001	4.08 ± 0.10	0.46 ± 0.227	$-224^\circ (-183^\circ, -251^\circ)$	<0.001	3.18 ± 0.11	0.36 ± 0.72	$-228^\circ (-212^\circ, -243^\circ)$
GR (nmol NADH Oxi/min/mg/protein)	<0.001	5.04 ± 0.10	0.83 ± 0.08	$-122^\circ (-117^\circ, -127^\circ)$	<0.001	3.43 ± 0.25	0.37 ± 0.08	$-143^\circ (-129^\circ, -158^\circ)$
Note: MDA: Malondialdehyde; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; CAT: Catalase, n: number of subjects, p: p-value from zero amplitude (norhythm) test; M: MESOR, a rhythm-adjusted mean value; A: 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								

As seen in Table 2, non-vegetarian stone patients had a higher MESOR ($\pm$ CI) of MDA (2.91 ± 0.077 vs. 2.31 ± 0.16 ;

$p < 0.001$), but a lower MESOR of SOD (16.03 ± 0.00 vs. 19.89 ± 0.42 , $p < 0.001$) as compared to non-vegetarian controls. Non-

vegetarian stone patients also had a lower MESOR of catalase (13.41 ± 0.30 vs. 14.39 ± 0.20 , $p < 0.001$), a lower MESOR of GPx (3.14 ± 0.14 vs. 4.02 ± 0.08 , $p < 0.001$) and a lower MESOR of GR (3.60 ± 0.02 vs. 5.00 ± 0.08 , $p < 0.001$) as compared to non-vegetarian controls. In Tables 3 and 4, parameter tests (tested jointly) indicate that the non-vegetarian diet-taking stone formers patients had a higher MESOR ($\pm$ CI) of MDA (2.88 ± 0.10 vs. 2.23 ± 0.22 and 2.91 ± 0.07 vs. 2.31 ± 0.16 ; $F=57.31$ vs. $F=39.36$, $p < 0.0001$). The parameter tests also indicate that the non-vegetarian diet stone formers patients had a lower MESOR ($\pm$ CI) of SOD (16.38 ± 0.31 vs. 19.88 ± 0.62 and 16.03 ± 0.00 vs. 19.89 ± 0.42 ; $F=346.09$ vs. $F=128.97$, $p < 0.0001$). It also had a lower MESOR of catalase (13.89 ± 0.32 vs. 14.56 ± 0.18 and 13.41 ± 0.30 vs. 14.39 ± 0.20 ;

$F=21.56$ vs. $F=6.32$). Similarly, parameter tests indicate (tested jointly) that the non-vegetarian diet stone formers patients had a lower MESOR ($\pm$ CI) of GPx and GR respectively (3.18 ± 0.11 vs. 4.08 ± 0.10 and 3.14 ± 0.14 vs. 4.02 ± 0.08 ; $F=114.87$ vs. $F=90.36$ $p < 0.001$) and MESOR ($\pm$ CI) of GR (3.43 ± 0.25 vs. 5.04 ± 0.10 and 3.60 ± 0.02 vs. 5.00 ± 0.08 ; $F=88.89$ vs. $F=61.73$, $p < 0.001$). The patients also differed from the healthy subjects in terms of their circadian amplitude and acrophase (tested jointly) for all.

Table 2. Circadian rhythm of plasma MDA, SOD, catalase, GPx, and GR in non-vegetarian renal stone formers and non-vegetarian controls.

Variable	Clinically healthy (k=30)				Stone formers non-vegetarian (k=50)			
	p-value	M $\pm$ CI	A $\pm$ CI	ϕ (95% CI)	p-value	M $\pm$ CI	A $\pm$ CI	ϕ (95% CI)
MDA (nmol/m)	<0.001	2.31 ± 0.16	0.48 ± 0.12	$-249^\circ (-227^\circ, -266^\circ)$	<0.001	2.916 ± 0.077	0.33 ± 0.08	$-208^\circ (-195^\circ, -225^\circ)$
SOD (units/ml RBCs)	<0.001	19.89 ± 0.42	1.43 ± 0.36	$-109^\circ (-96^\circ, -125^\circ)$	<0.001	16.03 ± 0.00	0.77 ± 0.23	$-118^\circ (-110^\circ, -131^\circ)$
Catalase (units/ml RBCs)	<0.001	14.39 ± 0.20	0.93 ± 0.16	$-120^\circ (-109^\circ, -130^\circ)$	<0.001	13.41 ± 0.30	0.75 ± 0.15	$-130^\circ (-120^\circ, -141^\circ)$
GPx (nmol NADH Oxi/min/mg/protein)	<0.001	4.02 ± 0.08	0.35 ± 0.13	$-181^\circ (-146^\circ, -219^\circ)$	<0.001	3.14 ± 0.14	0.40 ± 0.07	$-228^\circ (-215^\circ, -239^\circ)$
GR (nmol NADH Oxi/min/mg/protein)	<0.001	5.00 ± 0.08	0.68 ± 0.12	$-128^\circ (-118^\circ, -138^\circ)$	<0.001	3.60 ± 0.02	0.43 ± 0.06	$-145^\circ (-136^\circ, -154^\circ)$

Note: MDA: Malondialdehyde; SOD: Superoxide Dismutase; GPx: Glutathione Peroxidase; GR: Glutathione Reductase; CAT: Catalase, n: number of subjects, p: p-value from zero amplitude (norhythm) test; M: MESOR, a rhythm-adjusted mean value; A: 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 3. Comparison of circadian rhythm parameters between Vegetarian Stone Formers (VSF) and Vegetarian Controls (VC).

Variables	Populations	MESOR			Amplitude			24-h Acrophase (ϕ)			A, ϕ		
		DF	F	P	DF	F	P	DF	F	P	DF	F	P
MDA	NVC, NVSF	1.78	57.31	<0.0001	1.78	7.03	0.0097	1.78	9.5	0.0028	2.154	7.65	0.0007
SOD	NVC, NVSF	1.78	346.1	<0.0002	1.78	11.79	0.001	1.78	0.84	0.3601	2.154	5.59	0.0045
Catalase	NVC, NVSF	1.78	21.56	<0.0003	1.78	2.23	0.1386	1.78	1.72	0.1924	2.154	1.94	0.145
GPx	NVC, NVSF	1.78	114.9	<0.0004	1.78	1.11	0.2934	1.78	8.01	0.0059	2.154	3.85	0.023
GR	NVC, NVSF	1.78	88.89	<0.0005	1.78	15.25	0.0002	1.78	6.96	0.0101	2.154	9.15	0.0002

Note: 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 50 stone formers and 30 healthy controls; acrophase expressed in (negative) degrees with $360=24$ h and $0=00:00$; NS: Not Significant ($p > 0.10$)

Table 4. Comparison of circadian rhythm parameters between Non-Vegetarian Stone Formers (NVSF) and Non-Vegetarian Controls (NVC).

Variables	Populations	MESOR			Amplitude			24-h Acrophase (ϕ)			A, ϕ		
		DF	F	P	DF	F	P	DF	F	P	DF	F	P
MDA	VC, VSF	1.68	39.36	<0.0001	1.68	0.82	0.3679	1.68	13.48	0.0005	2.134	6.4	0.0022
SOD	VC, VSF	1.68	128.97	<0.0001	1.68	0.75	0.0002	1.68	0.287	0.5938	2.134	0.62	0.5393
Catalase	VC, VSF	1.68	6.32	0.0142	1.68	15.93	0.3679	1.68	5.78	0.0189	2.134	11.43	<0.0001
GPx	VC, VSF	1.68	90.36	<0.0001	1.68	1.39	0.2422	1.68	0.063	0.8011	2.134	0.97	0.3792
GR	VC, VSF	1.68	61.73	<0.0001	1.68	44.47	<0.0001	1.68	7.99	0.0062	2.134	20.51	<0.0001

Note: 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 50 stone formers and 30 healthy controls; acrophase expressed in (negative) degrees with $360^\circ=24$ h and $0^\circ=00:00$; NS: Not Significant ($p>0.10$)

Discussion

Our study of 100 patients with kidney stones revealed a clear redox imbalance-systemic lipid peroxidation was elevated (evident from significantly higher MDA), while key antioxidant defences (catalase, glutathione peroxidase and glutathione reductase) were suppressed. Reportedly, renal stone disease is associated with high oxidative stress and resultant damage to renal tubular cells [23,24]. MDA is a byproduct of free radical-induced lipid damage; its elevation indicates enhanced oxidative stress [25]. Catalase and GPx normally degrade Hydrogen Peroxide (H_2O_2) and lipid hydroperoxides, and glutathione reductase recycles Glutathione (GSH) to keep GPx active. Thus, the observed enzyme deficits imply that the patients' cells may accumulate H_2O_2 and other Reactive Oxygen Species (ROS), leading to greater cellular injury. Indeed, oxidative injury to renal tubular epithelium is thought to contribute directly to stone formation: Free radicals can peroxidise tubular cell membranes, causing cell death and shedding of membrane fragments that serve as nuclei for crystal aggregation [24]. Persistent ROS generation may damage the renal epithelium and expose cell debris on which calcium oxalate crystals can readily form stones [26]. ROS are also reported to amplify the inflammatory cascade associated with urolithogenesis. Crystal-induced cell stress upregulates ROS, which results in inflammation stimulating the release of cytokines. This fosters further tubular injury and facilitates adhesion and aggregation of crystals on injured epithelium [27].

A second, and more important, aspect that we have studied is the circadian rhythm of MDA, catalase, SOD, GR and GPx. It has been reported that the kidneys exhibit circadian rhythms, with functions such as urine volume, Glomerular Filtration Rate (GFR), sodium excretion, Renal Plasma Flow (RPF), urine protein excretion rate, urine electrolyte excretion rate, blood pressure, glucocorticoids, erythropoietin, endothelin, and others showing circadian variations [28]. We found a marked circadian variation in MDA concentration and catalase, SOD, GPx, and GR activity in healthy controls as well as stone formers. The circadian clock in our body is the key driver of many physiological aspects and maintenance of physiological homeostasis, with the kidneys playing a crucial role in this process.

Conclusion

In our study, we observed an altered circadian rhythm in stone formers, with a higher MESOR of MDA ($p<0.001$) than in healthy controls. Among stone formers, MDA was higher in vegetarians than in non-vegetarians. Contrary to earlier reports [29,30], we found plasma lipid peroxides to be higher in stone formers, both vegetarian and non-vegetarian, and a delayed circadian acrophase. To our knowledge, no literature is available regarding the circadian nature of lipid peroxides in stone formers (vegetarian vs. non-vegetarian), but it is clear that a role of lipid peroxidation and oxidative stress exists in the pathogenesis of urolithiasis.

The demonstration of a circadian rhythm in all the variables investigated herein suggests that these variables could also serve as putative markers to optimise the timing of drug administration and to assess the patients' response to the treatment. Further studies are needed to correlate the lipid peroxide concentrations with free-radical scavengers and their circadian rhythm after administration of dietary and therapeutic antioxidants in patients with renal stones.

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Conflict of Interest

None declared.

Ethical Approval

The study was approved by the Institutional Ethics Committee.

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