



## Diurnal Variation of Serum Urea , Uric Acid and Creatinine in Healthy Individuals

## KEYWORDS

Diurnal variation, ANOVA

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**ABSTRACT** *Objectives: The aim is to study diurnal variations of serum NPNs urea ,uric acid and creatinine in healthy individuals.*

*Study Design: observational & prospective*

*Place and Duration of Study: The study was carried out in Department of Biochemistry and department of Medicine, MGM Medical college, Navi Mumbai.*

*Methodology: Total 70 subjects comprising of male and female aged between 18 to 35 years were recruited in the study after obtaining informed written consent. The serum levels of urea, uric acid and creatinine were estimated three times a day at morning 8am, afternoon 1pm, and evening 6pm. The mean serum urea level, uric acid level and serum creatinine at various durations was compared using one way ANOVA.*

*Results: In our study, we found strong correlation of serum creatinine level after overnight rest morning fasting sample with sample collected in the afternoon and evening. Whereas, we have not observed any significant variation in the case of serum urea and uric acid levels.*

**INTRODUCTION**

A variety of biological variables oscillate within an organism including: behavior, physiological functions; and, biochemical factors. If any event within a biological system recurs at approximately regular intervals, it is generally referred to as a biological rhythm<sup>1,2</sup>.

Many of our biological and behavioral functions experience variations throughout the day, including: posture, sleep, body temperature, alertness levels and mental and physical performances. Many of these functions vary systematically in a cycle of about 24 hours and are called "circadian rhythms" (from the Latin words "circa" which means "about" and "dies" which means "a day").<sup>3</sup> These circadian variations are governed by a biological clock located in the brain. The mechanism underlying circadian rhythms is called the "biological clock" or the "circadian clock." Research has shown that the biological clock is located in the suprachiasmatic nucleus of the hypothalamus (a gland). The biological clock is probably the result of human evolutionary adaptation to the solar day<sup>4</sup>. Nitrogen containing compounds that are not proteins or polypeptides are called non protein nitrogen substances.

Present study has been carried out for diurnal variations for three major NPN components urea, uric acid, and creatinine which are routinely determined .in clinical settings .Finding of diurnal variations may help to assess whether these changes are of significant magnitude to require wider recognition and possible adjustment while interpreting clinical results.

**MATERIALS AND METHODS****Sources of the data**

The study was carried out in age group of 18-35 years

visiting medicine out Patient department of MGM Hospital, Navi mumbai .The present study was the observational prospective study, carried out in the Department of Biochemistry, MGM Medical College, Kamothe , Navi Mumbai study duration between period of Jan 2014 to Jan 2015.

**Sample collection**

**Blood sample:** In a plain vial, 4ml of blood sample from healthy volunteers were collected in the morning at 8:00am, afternoon 1:00pm and evening 6:00pm from each subject by veni puncture under strict aseptic technique. After clotting serum was separated for study.

**Inclusion criteria:** Healthy individuals between age group 18-35yrs were selected.

**Exclusion criteria**

- Chronic smokers
- Chronic alcoholics
- Individual with any diseases

Ethical clearance was obtained from Institutional Ethics Review Committee. (IERC).

All healthy volunteers were enrolled and a written informed consent was taken . The proforma included, name, age, sex, dietary habit Personal history of disease (if any), smoking habit, drinking habit, socio-economic status and occupation.

**Methodology:**

**Estimation of Serum Urea:** GLDH Urease method

**Estimation of serum uric acid:** Uricase POD method

**Estimation of serum creatinine:** Jaffe method

**RESULTS**

**Distribution According to Age (in Years)**

Age (in years)	Frequency	Percentage
18-22	14	20.0%
23-27	26	37.1%
28-32	21	30.0%
33-37	9	12.9%
Total	70	100.0%

**TABLE 1.** Mean Age 27.52 years, SD = 4.237 years, Min. = 18 years, Max. = 35years

The above table indicates descriptive statistics for age. Out of 70 study participants included in this study, 14 (20.0%) were in the age group 18-22 years, 26 (37.1%) were in the age group 23-27 years, 21 (30%) were in the age group 28-32 years and 9 (12.9%) were in the age group 33-37 years.

**TABLE 2. Comparison of urea at different time.**

		N	Mean	SD	SEM	F-stat	Df	p-value
Urea	08.00 am	70	22.771	4.990	.596	2.755	2,207	> 0.05
	01.00 pm	70	24.143	5.209	.623			
	06.00 pm	70	24.743	5.078	.607			

The mean urea level at 08.00 am was 22.771 (SD = 4.990). At 01.00 pm, the mean urea level was 24.143 (SD = 5.209) and at 06.00 pm, the mean urea level was 24.743 (SD = 5.078). The result indicates that there is no significant difference in the mean urea level at various durations (p >0.05)

**TABLE 3. Comparison of serum uric acid level at different time**

		N	Mean	SD	SEM	F-stat	Df	p-value
Uric Acid	08.00 am	70	5.626	.489	.058	0.016	2,207	> 0.05
	01.00 pm	70	5.636	.511	.061			
	06.00 pm	70	5.620	.574	.069			

The mean Uric acid level at 08.00 am was 5.626 (SD = 0.489). At 01.00 pm, the mean Uric acid level was 5.636 (SD = 0.511) and at 06.00 pm, the mean Uric acid level was 5.620 (SD = 0.574). The result indicates that there is no significant difference in the mean Uric acid level at various durations (p >0.05)

**TABLE 4. Comparison of serum creatinine level at different time ."**

		N	Mean	SD	SEM	F-stat	Df	p-value
Creatinine	08.00 am	70	.673	.075	.009	112.276	2,207	< 0.001
	01.00 pm	70	.847	.069	.008			
	06.00 pm	70	.807	.071	.008			

The mean Creatinine level at 08.00 am was 0.673 (SD = 0.075). At 01.00 pm, the mean creatinine level was 0.847 (SD = 0.069) and at 06.00 pm, the mean creatinine level was 0.807 (SD = 0.071). The result indicates that there is significant difference in the mean creatinine level at various durations (p < 0.001)

**DISCUSSION**

In present study serum levels of NPN urea, uric acid and creatinine of morning, afternoon and evening in healthy individuals were estimated. Changes in serum levels of urea,

uric acid and creatinine in these healthy individuals were analysed.

The present study includes total 70 healthy individuals. The serum levels of urea, uric acid and creatinine were estimated three times a day at morning 8am, afternoon 1pm, and evening 6pm respectively. Serum samples collected in the morning were fasting blood samples where as afternoon and evening blood samples were non-fasting. Comparison of each NPN during various durations were made. In this study time of day when the serum samples were collected has been considered an important factor in order to report findings of diurnal variations.

In our study conducted, serum urea levels were analyzed three times a day. The mean serum urea level at various durations was compared using one way ANOVA. The result indicated that there is no significant difference in the mean urea level at various durations (p>0.05). Further multiple serum urea comparisons were made for different durations that showed 7.75% increase in afternoon serum urea level compared to morning level. Whereas evening serum urea levels showed 2.39% increase compared to afternoon levels. These variations were not significant. When morning serum urea level was compared to that of evening 10.13% increase was observed.

Our findings for serum urea levels are in accordance with Eaton and Lois although not of such magnitude as to be of practical significance the blood urea concentration of normal individual undergoes fluctuations during the day<sup>5</sup>.

Our studies for serum urea levels are in accordance with Bernard E. Statland, Per Winkel, and Henning Bokelund. In their study serum urea ratios of serum values (1100 h/0800 h) on day of exercise showed insignificant increase at 1100 h compared to 0800 h<sup>6</sup>. Also, the mean values for serum urea sample collected at 1100 fasting and 1400 h after meal showed insignificant increase in their level<sup>6</sup>. We have observed a non significant increase in serum urea level that may be due to dietary protein which is in accordance with other studies.

For serum uric acid levels at various durations was compared using one way ANOVA. The result indicated that there is no significant difference in the mean uric acid level at various durations (p>0.05). Multiple comparisons showed 0.17% increase in afternoon serum uric acid level compared to morning. Whereas, there was 0.34% and 0.19% decrease in evening serum uric acid level compared to afternoon and morning serum uric acid level. At various time serum uric acid diurnal variations observed were not significant.

In the study carried out by Bernard E. Statland, Per Winkel, and Henning Bokelund serum uric acid level significantly increased at 1100 h after exercise compared to 0800 h before exercise<sup>6</sup>. Exercise we have not taken into consideration. Our studies has shown 0.17% increase in afternoon and these variations were not significant.

For serum creatinine levels at various durations were compared using one way ANOVA. The results indicated that there was significant difference in the mean creatinine level at various durations (p<0.001).

We observed 26.84% and 21.4% significant increase in serum creatinine level in afternoon and evening compared to morning serum creatinine. It may be due to the increase

in creatinine as the outcome of postural muscle activity, physical activity and energy output that is more in the afternoon compared to that during morning or evening. Also serum creatinine level showed 4.67% decrease during evening compared to that of the afternoon. This may be because of comparatively less postural muscle activity and increased glomerular filtration and its excretion by the kidneys. Though this decrease was not significant, upon using ANOVA method results obtained for serum creatinine comparisons for afternoon and evening showed significant variations.

Serum creatinine concentration depends upon the balance between the production of creatinine and its excretion by the kidneys. In addition serum creatinine concentration is related to glomerular filtration rate in a reciprocal fashion; when renal function is normal or only mildly impaired, small changes in serum creatinine concentration represent large changes in GFR<sup>7</sup>.

Our findings for serum creatinine are in accordance with S J Pocock et al. (1989) creatinine mean values were higher in the afternoon compared to morning and the diurnal trend was stronger in 25 healthy individuals<sup>8</sup>.

Our serum creatinine study are also in relation with Cordeiro N. And Friedman, M.H., serum creatinine measurements rise during recumbency and drop when erect lordotic posture is assumed<sup>9</sup>.

It is of interest to note that a drop in the postural muscle activity causes drop in vascular tension in normal individuals thereby decreasing serum creatinine formation following drop in the glomerular filtration rate<sup>10</sup>.

Study by T. Addis and colleagues are in relation to our findings that serum creatinine values decrease during rest hours or less muscle activity. Their study have inferred that there is a drop in serum creatinine during the period from 10 pm to 7 am and there was rise during the period from 7 am to 12 noon<sup>10</sup>.

This study was mainly carried out to report the findings of diurnal variations in 03 major NPN serum levels to assess whether these changes are of sufficient magnitude to require wider recognition and possible adjustment when interpreting clinical laboratory results.

The effect of time of the day while estimating serum NPN levels was seen in healthy subjects. One must consider diet, exercise, smoking, drug intake, and sleep as well. In the present study, significant diurnal increase in serum creatinine was observed whereas, the serum urea and uric acid levels showed non-significant diurnal variations in healthy individuals.

## CONCLUSION

In our study, we found strong correlation of serum creatinine level after overnight rest morning fasting sample with sample collected in the afternoon and evening. Whereas, we have not observed any significant variation in the case of serum urea and uric acid levels. Hence we recommend, to critically interpret serum creatinine load specially in CKD patients, because such patients are already having compromised kidney and so they might not be able to excrete the extra level of creatinine released due to postural muscle activity during day hours. This has to be confirmed by measuring NPN levels in larger number of CKD patients.

It is also suggested that while interpreting NPN levels in patients, clinicians must take in account the time of the day at which the sample is collected along with other factors like age, sex, diet, exercise, height, muscle, mass, etc.

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