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Influence of moon phases on serum level of sodium and lithium: a quasi-experimental study on rabbits

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The present research aims to study the effects of moon phases on serum sodium and lithium levels in rabbits. Twenty-four male rabbits in two separated groups (sodium and lithium groups) were studied. In each study group, there were 12 rabbits, and 40 mg/kg lithium carbonate was orally administered to rabbits in one group on a daily basis. On days 1, 5, 9, 13, 15, 17, 21, and 25 of a lunar month, blood samples were taken from all rabbits. The highest mean of serum sodium levels was observed on the first day and the lowest mean was related to the 15th lunar day. Significant difference was noted between the 1st, 5th, 13th, and 17th days of the lunar month. In the lithium group, there was also a decreasing trend from the 1st to 17th lunar day in serum lithium level, but there was no statistical difference between lithium serum levels on different days of the lunar month. Fluctuations of bodily fluids under the influence of moon phases can be an effective factor in the changes in serum sodium levels. It is probable that due to small sample size we could not find significant difference in serum lithium levels.

Keywords: moon; serum; sodium; lithium; rabbit

1. Introduction

Literature review about the effects of the moon on the physiology and behavior of the vital organisms shows that global multidisciplinary investigations in this area have been significantly developed in recent decades. However, there are some debates about these effects and their extent and quality. Effects of different moon phases on some behavioral and physiological aspects include those of fertility, menstruation, and births (Cutler 1980; Criss and Marcum 1981; Guillon et al. 1986; Law 1986; Ghiandoni et al. 1997), hospital admissions (Payne et al. 1989; Sitar 1989; Mikulecky and Schreter 1993; Oomman et al. 2003; Roman et al. 2004), and accidents, violence, and suicides (Jones and Jones 1977; Lieber 1978; Thakur and Sharma 1984; Alonso

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1993). The authors had previously reported that most suicides and psychiatry hospital admissions were during the full moon period and the lowest rates were related to the period between new moon and full moon. Also the highest and lowest occurrences of the car accidents were related to the full moon and new moon periods, respectively (Malek and Dastgiri 1996). However, normal vaginal deliveries mainly occurred in the new moon and full moon periods; the new-full moon and full-new moon intervals had lower occurrences (Malek et al. 1999).

On the other hand, some other researches have shown that moon phases have no effect on the frequency of births (Waldhoer et al. 2002; Arliss et al. 2005; Morton et al. 2005), psychiatry hospital admissions (Robert et al. 2006), emergency case incidences (Wolbank et al. 2003; Zargar et al. 2004), cardiopulmonary arrest (Alves et al. 2003), survival of breast cancer patients (Peters et al. 2001), and quality of surgical operations (Holzheimer et al. 2003).

These controversies may be due to some methodological considerations especially in human research, which will be discussed later in this article. This would demonstrate the necessity for conducting methodologically respected investigations as well as animal studies.

Numerous researches have been developed in various evolutionary stages in animals including insects, fishes, reptiles, birds, and mammals such as mice and grey squirrels that demonstrated some physiological changes congruent with lunar phases (Zimecki and Webb 1976; Zimecki and Wieczorek 1991; Mikulecky and Bounias 1997; Pavlova 1997; James et al. 2000; Rahman et al. 2003, 2004; Takemura et al. 2004). These physiological changes congruent with lunar phases are not yet expressive of a unique or specific mechanism relevant to the association between the moon and organisms.

We assumed that one probable mechanism is fluctuations in body fluid levels congruent with moon phases, and therefore the serum sodium and lithium levels in male rabbits in different moon phases were evaluated. In animals with closed body spaces, the moon is less likely to affect the total fluid volume, but the fluids may be transfused via semi-permeable membranes and result in changes in serum sodium level and osmolarity in body fluids. Also, despite most psychotherapeutic drugs, the lithium ion (Li^+) is not lipophilic, has no plasma protein binding, and its final distribution volume is relatively similar to that of total body water (Joel and Lee 2001). Regarding the near association between serum lithium level and total body fluid volume, this study may develop new horizons in quantitative researches for the evaluation of effects of moon on animals' and humans' body fluids. Also evaluation of the effects of different lunar phases on serum lithium levels in male rabbits would result in some ideas for causes of recurrence or exaggerations of symptoms in those patients consuming lithium. A review of the available literature showed no study about fluctuation in serum sodium and lithium levels according to different lunar phases.

2. Methods and materials

This quasi-experimental study was performed in two groups of 12 white male rabbits from the New Zealand race (sodium group and lithium group). During the study, due to some problems blood sampling from the ear, three rabbits were excluded from each group and finally nine rabbits were evaluated in statistical analysis in each group.

The animal procedures were in strict accordance with the National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Utilization Committee. The rabbits (2.20 ± 0.4 kg) were housed singly in the animal room with an automatic temperature (22°C) and lighting (12-hour light–dark cycle) control. An adaptation period of 2 weeks for vehicle (tap water) administration was allowed before the initiation of the experimental protocol. A total of 6 weeks of experiments were planned. The rabbits were allocated to normal rabbit chow diet with water alone (sodium group) or with additional oral lithium carbonate (Lithium group = oral 40 mg/kg/day) ($n = 9$, each group). In the lithium group, lithium was given daily for 1 week prior to blood sampling and during the total course of the study. After 1 week, when the serum lithium level reached a steady state, the bioavailability of this method was assessed by sampling from two rabbits and estimation of serum lithium level.

The blood sampling from ear marginal vein was performed in days 1, 5, 9, 13, 15, 17, 21, and 25 of the lunar month of Ramadan 1429 A.H. (September 2008). The number of selected days and the intervals were due to total permitted blood volume that may be sampled from a rabbit during a month. Due to alterations in times of maximal tidal flow in different days, the samplings were performed according to a special time schedule and in times of maximal flow in the geographical region of Tabriz. So, regarding this key methodological point, the blood sampling hours and the lithium administration times differed diurnally and lithium was persistently administered 10 hours before sampling (Table 1).

Since the nutrition is effective in serum sodium and lithium levels and the water and electrolyte fluctuations would reach a steady state after 2–3 hours of nutrition, the water and food were ceased since 3 hours prior to sampling (Table 1).

The sodium level measurement was performed by Flame photometer (Digiflame) and the serum lithium level was measured using digital Flame photometer (Corning 506).

Data from the rabbits were analyzed using SPSS (version 15.0) software (Statistical Procedures for Social Sciences; Chicago, Illinois, USA). Differences were tested by calculating the mean and confidence interval for serum sodium and lithium levels on different days.

Table 1. Time schedule for blood sampling of rabbits in different lunar days based on the maximal flow hour of each day.

Lunar days (Ramadan 1429)	Solar days (September 2008)	Sampling time (maximal flow hour of the selected day)	Time of food and water cessation	Time of lithium administration
1	2	02:28 PM	11:28 AM	04:28 AM
5	6	06:23 PM	03:23 PM	08:23 AM
9	10	09:46 PM	06:46 PM	11:46 AM
13	14	12:07 AM	09:07 PM	02:07 PM
15	16	01:39 AM	10:39 PM	03:39 PM
17	18	03:17 AM	12:17 AM	05:39 PM
21	22	07:12 AM	04:12 AM	09:12 PM
25	26	11:07 AM	08:07 AM	01:07 AM

3. Results

3.1. Sodium group

The highest and the lowest mean serum sodium levels were seen on the 1st and 15th days of lunar month, respectively. As shown in Figure 1, the mean serum sodium levels on the 1st (148.2 ± 2.6, 95% CI = 145.6–150.8), 5th (142.6 ± 1.5, 95% CI = 141.1–144.1), 13th (141.5 ± 2.9, 95% CI = 138.6–144.4), and 17th (141.1 ± 3.7, 95% CI = 137.4–144.8) lunar days had no overlap showing a significant difference between serum sodium level on the 1st day and those of days 5, 13, and 17.

3.2. Lithium group

The lowest and the highest mean serum lithium levels were seen on the 17th day and 5th and 25th days of lunar month, respectively. There was a decreasing trend in serum lithium level from 1st to 17th lunar days. As shown in Figure 2, the mean and confidence interval of serum lithium levels in all lunar days have overlap showing no significant difference between serum lithium levels in different days.

4. Discussion

The results of this study showed that there is a significant difference between serum sodium levels on the first day and those on days 5, 13, and 17 with a decreasing trend in serum sodium level from the first lunar day up to full moon days. A similar trend was observed for serum lithium level; but with no significant difference between various days.

There are no similar studies for comparison in land mammals. Only one study in marine animals showed that in a fish species, i.e. *Siganus argenteus*, which spawns

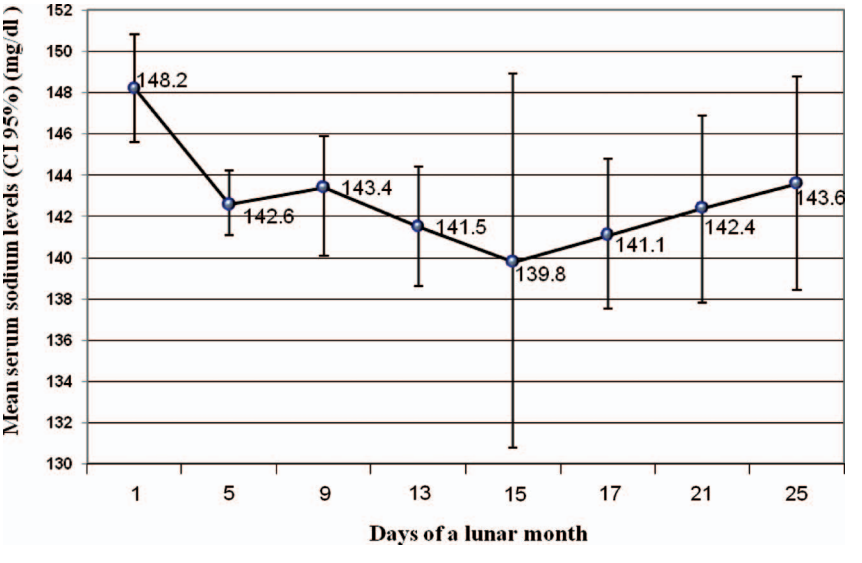

Figure 1. Mean serum sodium levels (95% CI) in different lunar days in rabbits in sodium group.

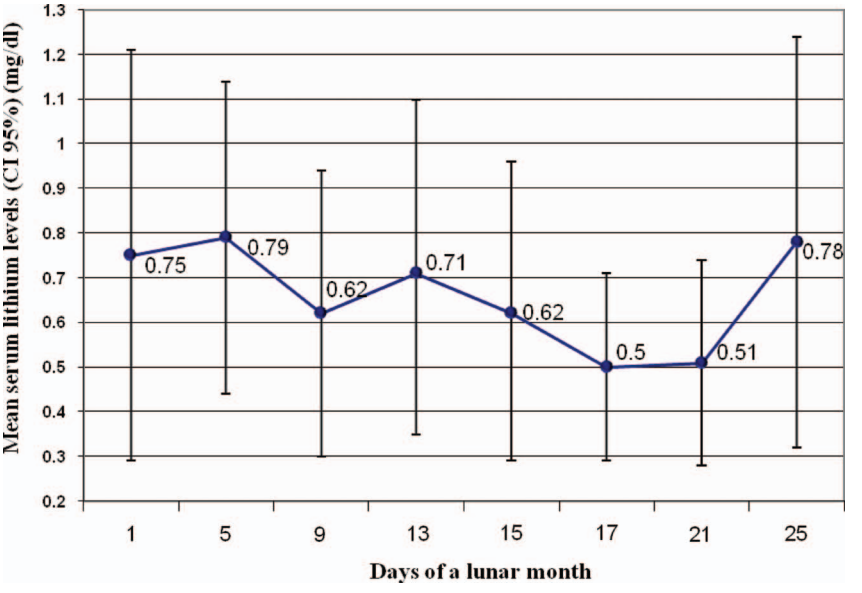

Figure 2. Mean serum lithium levels (95% CI) in different lunar days in rabbits in lithium group.

synchronously around the last quarter moon, the osmolarity of seminal fluid decreased in that period (Rahman et al. 2003).

The effects of moon during its turning around the earth have been considered in relation to monthly biological rhythms (circatrigintan). Nowadays, the rhythmic changes are believed to be the essential characteristics of life. All living beings ranging from unicellular creatures to human beings are performing with a rhythmic pattern. The range and spectrum of the rhythms are extended and may differ in range from ultradian, circadian, infradian, circaseptan, and circatrigintan to circanual.

There have been debates about the genetic or acquired basis of biological rhythms. But the acceptable theory is the genetic basis with fluctuation according to environmental changes. Biological rhythms, indeed, are a reminder of predictable rhythmic environment and "the obvious physical cycles to which a person's biological rhythms conform include the day–night cycle, the lunar month, the solar year, and biophysical constraints." (Sadock and Sadock 2007). Hence, despite an originally independent nature of the biological rhythms from environmental or exogenous factors, the changes in these factors may result in change in rhythmic activities.

Depending on the status of the moon in relation to the position of the sun and the earth, there are two occasions that the sun, the moon and the earth would be in the same direction in a lunar cycle. This would result in an increase in the gravitational pull of the sun and the moon on the earth. This situation is seen in new moon and full moon periods. This would result in an aggregation of some physical phenomena on the earth namely the tide in the seas and oceans. Monthly, on the 1st and 14th days, the tide extent is at the maximal state. These effects may be extended to biological phenomena leading to a biological tide due to moon gravity that may result in some physiological and biochemical changes in human body with water consisting more than 50% of the human body. In addition to gravity, some other

mechanisms such as moon light and changes of earth magnetic field due to the moon's turning around the earth may be effective and evaluation of their role as a whole or separately is an interesting research area.

There is no definite mechanism explaining the cause of fluctuations in serum sodium level congruent with the changes in moon phases found in this study. But as previously mentioned, the fluctuations in body fluid and transition of intra- and extracellular fluids resulted from moon phases may be the contributing factor. The presumed cause about the lack of significant difference between serum lithium levels in this study may be small sample size. However, as mentioned in the 'Introduction', some researchers have been unable to show any significant difference between moon phases and some physiological and behavioral phenomena in humans (Peters et al. 2001; Waldhoer et al. 2002; Alves et al. 2003; Holzheimer et al. 2003; Wolbank et al. 2003; Zargar et al. 2004; Arliss et al. 2005; Morton et al. 2005; Robert et al. 2006). Our experience and ideas arising from different research in the fields demonstrate that this matter may have some methodological causes that respecting them would help the researchers in these fields:

- The sample size may be small in comparison with those studies that approved this association (such as fluctuations in serum lithium levels in our study).
- The effects of the moon in various geographical locations are different.
- The moon turns around the earth in an oval circle, and when it is in the perihelion the forces resulting in tide are at the maximal state with a 20% more force than the usual conditions. Hence, with regard to the moon being in the peak or the perihelion at the full moon or new moon phases at the time of the research, the results may differ.
- The subject-to-study phenomena may have essentially been independent of moon phases, but the authors have gained a wrong primary assumption.
- Some phenomena may be under the effects of moon light, some under the influences of moon gravity, and some under the effects of earth magnetic fields due to lunar cycle. Hence, when the matter is seen descriptively, different results may be observed about different behavioral and physiological phenomena in relation to different days of lunar months. It means that based on the type of mechanism involved in the association between moon and specific behavioral or physiological phenomenon, a phenomenon may fluctuate in full moon and other phenomena in new moon or other phases.
- One of the most powerful factors effective in the regulation and synchronization of biological rhythms in human beings is the socio-ecologic condition that may affect the natural status of biological rhythms. This matter may be seen in association with lunar cycle as a natural environmental synchronizer and monthly rhythms in human beings, so that the socio-ecologic status of human beings may make the pattern of monthly rhythms incongruent with lunar phases. However, socio-ecologic status may also be effective in animals as an exogenous synchronizer, but it would act with more complex, effective, and rigorous characteristics in human beings (due to special social conditions of human life). Hence, the animal studies in monthly rhythms may reflect more definitely the fluctuations in monthly rhythms in association with lunar phases.
- The gender differences in association with lunar phases and some phenomena in human beings may be important. Totally, considering the physiological and hormonal differences between males and females, some monthly fluctuations congruent with moon phases may be due to gender differences.

The results obtained in this study need to be repeated with a larger sample size. Approval of significant positive findings of this study in following research may be an effective step for developing more objective methods for study of the effects of the moon on living beings' behaviors. Controlled trials, despite the complexity due to confounding factors, in human beings are recommended.

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