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ALTERATION OF IMMUNOGLOBULIN AND TEMPERATURE RHYTHM DURING WINTER-OVER STAY IN ANTARCTICA

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Abstract

The alien photoperiodicity of Antarctica can disrupt the biological clock, thereby desynchronizing the various bodily rhythms, which can have health implications. This study is an attempt to find out whether the circadian rhythm disruption could be a reason for the immunosuppression observed during Antarctic stay. In the present study, in one group of six volunteers, the immunoglobulin rhythm was assessed. In another group of six volunteers the oral temperature, activity and heart rate rhythms were measured. Disruption and desynchronization of the temperature rhythm with respect to activity and heart rate rhythms was noted. In the other group, disruption of the serum immunoglobulin rhythm was noted irrespective of the season. Our results clearly indicate that the decrease in the serum immunoglobulins IgG and IgM are associated with disruption of their circadian rhythm. Involvement of neuroendocrine factors is postulated.

Introduction

More than 100 years back Reinert (1891) reported the circadian rhythm of leukocyte counts in humans. Following which there are many reports to show that different compartments of the immune system have different individual rhythms (Haus *et al.,* 1983; Gatti *et al*., 1987; Fernandes *et al,* 1976). Amongst the many zeitgebers that tell the milieu interior about the time of the day, the photoperiodicity is the most potent external cue (Czeisler, 1995).

In Antarctica there is extreme cold coupled with an alien photoperiodicity of long polar days and polar nights amongst many other physical and social stressors, which a human sojourner has to face while on duty in Antarctica. The disturbances of hormonal and circadian sleep-wake cycle (Kennaway and Von-Dorp, 1991) have already been reported in Antarctic sojourners.

Though there are many studies to show the immunosuppression during Antarctic stay, there are no reports to show whether any circadian component

is involved in modulating the immune status of humans while in Antarctica. In this study we measured the circadian rhythm of serum immunoglobulin IgG and IgM and found that the circadian rhythm of immunoglobulin is disrupted during prolonged stay in Antarctica. This study is the first report to show the disruption of human immunoglobulin rhythm during their stay in Antarctica on winter-over assignment.

Materials and Methods

Study Subjects

The human subjects included in this study were all Indian, male members of the 15th Indian Scientific Expedition to Antarctica who were on winter-over assignment. The objectives of the study were briefed to all the members of the expedition and only those who volunteered for the study were included. Out of the 25 winter-over members two groups of 6 volunteers each were included in the study. Group-I had a mean age of 33.51 ± 5.42 years and mean body weight 68.47 ± 6.25 kg in whom the circadian rhythm of body temperature, activity and heart rate were studied. In Group-II, with mean age 34.17 ± 2.73 years and mean body weight 67.17 ± 7.22 kgs, the circadian rhythm of immunoglobulin was studied.

All the subjects in the study were volunteers only. The ethical considerations set by the Ethical Committee of All India Institute of Medical Sciences were strictly followed. The subjects were given the freedom to drop out at any point of the study, if they felt so.

Periods of the Study

The study was conducted in six phases as given below:

Phase I - Basal : During onward voyage on board ship in December 1995, underbasal conditions, before exposure to Antarctic conditions (23°S, 56°E, L/D 12:12, 24 ±2°C).

Phase II - Early Winter : In April 1996 in Antarctica in early winter, at the beginning of isolation while the hours of darkness were increasing everyday (L/D 10:14;-7.9°C).

Phase III - Mid Winter: In July 1996, before the end of two months continuous darkness (L/D 1:23; -11.8°C).

Phase IV - End Winter : In October 1996, after the end of winter darkness and maximum cold (L/D 14:10; -8.3°C).

Phase V - Summer : In December 1996, during peak summer just before the end of ten months of isolation **(L/D** 23:1; -0.2 °C).

Phase VI - Post Exposure (Recovery) : On board ship during the return voyage, after exposure to Antarctic summer conditions during March 1997 (26°S, 54°E; LTD 13:11;+26°C).

Experimental Procedures

Blood Sampling for Circadian Rhythm Assessment of Immunoglobulins

Venous blood samples were collected, at four hourly intervals (1800, 2200, 0200, 0600, 1000 and 1400 hrs. local time) from the ante-cubital area using 5-ml syringe and 22G needle. All the blood samples were left for two hours to clot and thereafter, the serum was separated by centrifugation at 1500 rpm for 15 min. and stored frozen at -20 °C in a deep freezer. All estimations were done after return to New Delhi. Repeated thawing and freezing was avoided.

*Enzyme Linked **Immunosorbant** Assay (ELISA) of **Immunoglobulins***

The method of Kaur *et al.,* (1991) was adopted. The standard Immunoglobulin G or Immunoglobulin M (DAKO) or the test sera optimally diluted in 0.5 M Carbonate Bicarbonate Buffer (pH 9.6) were coated in triplicate wells on to the solid phase (COSTAR ELISA plates, 96 well, flat bottom) and incubated at 37 °C for 4 hours in a humid chamber. After decanting and washing with Phosphate Buffered Saline (PBS, 0.1 M ; pH 7.25) non specific binding was blocked using skimmed milk powder (Anikspray) by incubating at 37 °C for one hour. Following PBS-Tween washing, Anti Human Ig-HRP conjugate (DAKO) was then added and incubated for one hour at 37 °C. The chromogen (Ortho Phenylene diamine dihydrochloride [OPD], Sigma) dissolved in the Substrate buffer (1.5 M citrate phosphate buffer; pH 5.0) was used to develop the color and the reaction was stopped using 8N H_2SO_4. The optical density was then read in an ELISA reader, (Pharmacia) at 490 nm.

Circadian Rhythm of Temperature, Activity & Heart Rate

The circadian rhythm of temperature, activity and heart rate were measured using the ambulatory Minilogger-2000 (Mini Mitter, Sun River, USA) equipment. The Minilogger-2000 is a small battery operated computer. It is designed to acquire and store temperature, activity and heart rate as digitized signals and later to download the data to a host PC through a serial interface. The temperature and activity data are collected using specific sensors, which are positioned in the body and connected to the Minilogger through a cable. The heart rate is

measured by using a chest band, which picks up the electrical activity of the heart and relays the signal to Minilogger, by telemetry.

Temperature Sensors

Sheridan Sher-I-Temp (Sheridan Catheter Corp., USA) Tympanic Cavity Temperature Sensors were used. These have an accuracy of measuring upto a resolution of ± 0.05 °C. These temperature probes come equipped with a protective foam covering over the sensor end of the unit. This holds the sensor in place and protects the delicate tissues of the ear canal from injury. Care was taken to avoid contact with the tympanic membrane. The temperature measured at every hour interval was taken for analysis.

Activity Sensor

Wristband activity sensors were used. This was strapped onto the dominant hand of the subject and connected to the Minilogger-2000 through a cable. 'These sensors are 'omni-directional' using mercury switches. The activity was measured as the total counts in the sampling time interval.

Heart Rate Sensors

The Polar heart rate pickup belts (Polar Electro Oy, Finland) has a pair of conductive pads molded into a central transmitter unit and an elastic band to go around the chest, holding the transmitter in place. Since these pick up the electrical activity of the heart, ECG accurate heart rate is measured using this system. Heart rate data are reported to the nearest whole beat per minute. The heart rate was measured at hourly intervals.

Statistical Analysis

The circadian rhythm data were analyzed using the cosinar analysis (Nelson *et al;* 1979) to obtain the circadian parameters viz., Percentage rhythm (PR), Mesor (M) the rhythm adjusted mean; Amplitude (AMP) half the total variability between peak and trough; Acrophase (ACRO), peak time with mid-night as arbitrary phase reference. Each, rhythm was individually analyzed and the resultant circadian parameters were pooled and their mean was obtained. Paired 't' test was used to find out the differences between two.group means.

Results

C*ircadian Rhythm of Immunoglobulin G*

The results of the serum IgG in four hourly samples showed that many of the circadian rhythm parameters were disrupted following exposure to Antarctic conditions. These results, which are summarized in **Table-1,** shows that the mean percentage rhythm of six subjects was significantly ($p<0.01$) decreased during the end winter phase. However the mesor was decreased in all the phases of the Antarctic stay, but was significantly lower ($p<0.05$) only during the early winter phase and during the summer phase which was measured just before the end of the 10 month long isolation period. But most interesting results were observed in the amplitude of the rhythm, which was significantly decreased in all the phases of the study starting from the early winter phase. There was no consistency in the timing of the acrophase also, which showed statistically significant phase delay during the early winter and return voyage phases.

Table 1: Circadian Rhythm of IgG

Parameters	Basal	Early Winter	Mid Winter	End Winter	Summer	Return
PR (%)	56.72 ± 11.80	79.59 ± 18.53	61.19± 23.40	22.19 ± 19.84 a2	35.73 ± 27.03	40.96 ± 29.79
MESOR (mg/dL)	1405.38 ± 175.52	1197.00 ± 65.96 al	1295.02 ± 68.80	1282.34 ± 62.10	1220.34 ± 39.80 al	1329.24 ± 46.50
AMP (mg/dL)	377.40 ± 213.96	157.24 ± 36.34 al	93.03± 47.54 a2	56.40 ± 45.98 a2	59.12 ± 26.56 a2	71.94 ± 43.46 a2
ACRO (hrs)	22.53 ± 3.19	3.52 ± 1.3 a2	00.74 ± 4.14	20.99 ± 2.24	18.63 ± 5.84	02.76± 1.29 a1

a = In comparison with basal; 1=$p<0.05$, 2=$p<0.01$, 3=$p<0.001$

Circadian Rhythm of Immunoglobulin M

In comparison with IgG, the circadian rhythm of IgM was less affected. There were a significant reduction in the mesor and amplitude during the mid winter phase alone with the values being comparable to the basal values in the rest of the phases. The percentage rhythm was comparable to the basal values in all the other phases studied. The timing of the acrophase was also consistent throughout the period of stay in Antarctica, however there was a significant phase delay during the return voyage. These results are shown in **Table-2.**

Table 2: Circadian Rhythm of IgM

Parame-ters	Basal	Early Winter	Mid Winter	End Winter	Summer	Return
PR (%)	51.44 ±15.60	38.34 ± 23.19	37.10 ±36.72	31.66 ± 30.96	52.92 ± 20.83	69.89 ± 12.85
MESOR (mg/dL)	109.26 ± 30.84	103.11 ± 37.32	68.19 ± 26.52 al	102.15 ± 51.93	85.92 ± 35.40	130.20 ± 46.53
AMP (mg/dL)	19.17 ± 6.48	13.17± 16.83	6.51 ± 3.18 a2	13.56 ± 11.28	16.44 ± 7.41	28.26 ± 15.45
ACRO (hrs)	16.76 ± 5.19	16.44± 3.62	17.52 ± 3.84	15.24 ± 1.80	20.53 ± 4.83	23. l0 ± 2.72 a l

a = In comparison with basal; l=p<0.05,2=p<0.01,3=p<0.001

Temperature Rhythm

The temperature rhythm (Table-3) was affected only in terms of its percentage rhythm, while the mesor and amplitude were unaffected. The percentage rhythm remained significantly lower than the basal throughout the study period in Antarctica. It became comparable to the basal during the return voyage. The acrophase was phase advanced during the winter phases, though significantly only during the early winter phase. During the summer and return voyage phases the acrophase however became adjusted and was comparable to the basal.

Table 3: Temperature Rhythm

Parameters	**Basal**	**Early Winter**	**Mid Winter**	**End Winter**	**Summer**	**Return**
PR(%)	46.81 ± 17.18	17.14± 14.72 a2	24.44 ± 13.69 **al**	11.11 ± 8.44 a3	23.44 ± 9.33 **al**	32.77 ± 24.02
MESOR (°C)	34.98 ± 0.86	34.97 ± 0.48	35.04 ± 0.32	34.75 ± 0.64	35.01 ± 0.36	35.46 ± 0.38
AMP (°C)	0.57 ± 0.26	0.31± 0.21	0.33 ± 0.13	0.33 ± 0.19	0.36 ± 0.09	0.33 ± 0.17
ACRO (hrs)	18.54 ± 2.43	12.50 ± 5.87 **al**	13.23 ± 6.39	15.18 ± 4.98	18.79 ± 2.70	17.04 ± 1.87

a =In comparison with basal; l=p<0.05, 2=p<0.01,3=p<0.001

Activity Rhythm

Unlike the temperature rhythm, the activity rhythm was not affected during winter-over. As shown in **Table-4,** none of the circadian parameters were affected during the entire period of winter-over stay, despite a very slight non-significant decrease in the mesor and amplitude during the mid **winter** phase.

Table 4: Activity Rhythm

Parame-ters	Basal	Early Winter	Mid Winter	End Winter	Summer	Return
PR (%)	33.27 ± 27.00	42.51 ± 18.86	32.87 ± 13.71	51.88 ± 16.15	49.01 ± .7.23	45.51 ± 10.17
MESOR (counts)	1201.33 ± 788.79	1184.00 ± 517.52	940.00 ± 317.35	1023.17 ± 288.34	1273.25 ± 208.43	1022.17 ± 118.88
AMP (counts)	909.00 ± 879.84	705.20 ± 358.90	664.50 ± 346.10	969.17 ± 390.85	1048.75 ± 320.17	975.67 ± 259.10
ACRO (hrs)	16.00 ± 1.70	15.55 ± 2.06	17.34 ± 1.05	17.48 ± 1.82	14.73 ± 1.29	17.87± 2.59

a = In comparison with basal; l=p<0.05, 2=p<0.01, 3=p<0.001

Heart Rate Rhythm

Similar to the activity rhythm, the heart rate rhythm was also unaffected during the winter-over stay. The only significant change noticed was a significant phase advancing of the acrophase during the summer phase at the end of the isolation. During this phase the acrophase phase advanced to 15.14 ± 1.10 hours from the basal value of 17.16 ± 1.62 hours (Table-5)

Table 5: Heart rate Rhythm

Parame ters	Basal	Early Winter	Mid Winter	End Winter	Summer	Return
PR (%)	41.52 ± 20.58	41.70 ± 21.16	43.12± 22.14	57.91 ± 8.88	55.45 ± 10.91	56.25 ± 19.78
MESOR (bpm)	79.84 ± 10.20	80.29 ± 7.00	73.77 ± 8.46	74.13 ± 7.75	80.51 ± 6.49	71.51± 7.40
AMP (bpm)	12.01 ± 4.21	18.94 ± 12.42	10.87 ± 5.26	13.74 ± 3.22	13.73 ± 3.24	15.16± 4.65
ACRO (hrs)	17.16± 1.62	16.91 ± 0.96	17.10± 2.39	17.96 ± 1.85	15.14 ± 1.10 a1	16.51 ± 4.09

a = In comparison with basal; l=p<0.05, 2=p<0.01, 3=p<0.001

Discussion

With prolonged winter stay there was derangement of immune rhythm. The amplitude suppression of IgG indicates direct control of circadian oscillator on lymphocyte function that controls the stimulation and release of IgG. Since the amplitude decreased irrespective of season, it could not be a direct effect of photoperiodicity. The stress of Antarctic stay and associated neuro-hormonal factors could be a possible reason for this.

In the present study, we did not attempt to correlate the psychological changes with circadian changes, although the psycho-immunological concept for immunomodulation under various life situations have been documented (Ader, 1981). The phase shift by primary oscillator governs all the physiological rhythms including immunoglobulins. Psychological disturbances (Lewy *et al;* 1987; Czeisler *et al,* 1989) related to abnormalities in pacemaker in its timing due to the stresses of Antarctica might be responsible for loss of immunoglobulin rhythmicity, which is evidenced by the blunting of circadian amplitude.

The acrophase of the IgG rhythm showed a phase delay during onset of winter and progressively phase advanced with the crossing of mid winter and into the following summer. Here increasing hours of darkness seems to be having a phase delaying effect on the timing of acrophase of IgG rhythm.

Thereby in Antarctica, there could be factors other than photoperiod involved in modulating the circadian rhythm of IgG, and the effect of photoperiod may be limited only in changing the timing of acrophase and thereby possibly the period of the rhythm.

The temperature rhythm was significantly disrupted during the entire period of Antarctic winter stay associated with internal desynchronization with respect to activity and heart rate rhythms. Under basal conditions, as seen in this study or other studies (Motohashi *et al,* 1987; Motohashi, 1990; Reinberg *et al,* 1989), the acrophase of temperature rhythm follows peak of activity and heart rate rhythms. During prolonged Antarctic winter-stay the phase advancement of temperature rhythm and a slight phase delay in activity and heart rate rhythms resulted in internal desynchronization. Such internal desynchronization is associated with intolerance to shift work (Pati and Saini, 1991; Reinberg *et al,* 1988) or can be present even in normal healthy individuals with just a mild irregular sleep-wake habit (Motohashi, 1990). Sleep disturbances in Antarctic stay have already been reported (Natani *et al,* 1970). The desynchronization that was observed in this study might be associated with the irregular sleep-wake cycles during Antarctic stay.

Gander *et al.* (1991) observed phase delays in the temperature rhythm during Antarctic summer. Such a phase delay was seen in the summer phase of the present study also. However phase advancement of temperature rhythm during Antarctic winters was observed in the present study. During the following summer at the end of the isolation period however the temperature rhythm phase delayed in comparison to the previous (end winter) measurement but not significantly different to that of the basal. Hence it is possible that certain neuroendocrine or behavioral mechanisms associated with prolonged period of light is involved in phase delaying the temperature rhythm of humans in Antarctica. Like wise factors associated with prolonged periods of darkness are implicated to have an opposite effect.

According to Motohashi *et al.* (1987) the phase advancing is usually associated with a rhythm period shorter than 24 hours. Since in the present study there was a phase advancing present in the temperature rhythm with no corresponding change in the activity or heart rate rhythms, it can be postulated that the period of the temperature rhythm alone was non-circadian in Antarctica.

In the present study, the internal desynchronization has been described as a change in the timing of the acrophase only, due to the fact that only 24 hour recording was possible in the present study. A longitudinal study of about 10 days or more would have given a much better picture in detection of the internal desynchronization (Reinberg *et al.,* 1989).

In the light of the above findings it becomes clear that the immunosuppres sion observed in Antarctica could be due to the alterations of the functioning of the biological clock.

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