Circadian Rhythm of *Wuchereria bancrofti* Microfilariae in Human Subjects in Raipur city, Chhattisgarh state, India

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The presence of *W. bancrofti* microfilariae in the peripheral blood of infected human in 24-hour periodic cycle is synchronized with the biting habits of the mosquito vector in the area. In this context an attempt was made to look into the rhythmic behavior of microfilariae emergence of *W. bancrofti* in human subjects on 24-hour scales at Raipur. A significant circadian rhythm in emergence of microfilariae in peripheral blood was recorded in all microfilaraemic subjects.

**Introduction**

Biological rhythms are ubiquitous among living organisms. It is presumed that organisms have developed various endogenous rhythmicities during the course of their long evolutionary history. Biological rhythms, thus, have some adaptive value. In the above context temporal organization in *Wuchereria bancrofti* is of immense importance and concern. The accumulation of *W. bancrofti* microfilariae in the peripheral blood of infected humans follows a 24-hour periodic cycle which appears to synchronize with the biting habits of the mosquito vector in the area [1,2]. Filarial larvae exhibit a remarkable rhythm in their appearance in the peripheral circulation. The periodicity shown by the microfilariae (mf) of lymphatic filarial worm, *W. bancrofti*, is classified into three sub-groups namely nocturnally periodic, nocturnally sub periodic and diurnally sub periodic [3]. The nocturnally periodic type shows a marked peak in mf density during the night hours; very few are found during the day in blood taken for routine examination. In nocturnally sub periodic and diurnally sub periodic types mf are found in the peripheral blood during the 24-hours cycle with one or more peaks. In South East Asian countries, *W. bancrofti* is generally known to be nocturnally periodic with peak in mf density between 22:00 and, 02:00 [4]. Sub periodic form of *W. bancrofti* has been reported in India and Thailand [5]. In the Western Pacific region of the World, periodic as well as sub periodic behavior of microfilariae emergence has been observed. The periodicity in mf emergence has been observed in China, Malaysia, Philippines, Papua New Guinea and Vietnam. However, the sub periodic nature of *W. bancrofti* mf has been prevalent in western Pacific and is very common in American Samoa, Cook Island, Fiji, French Polynesia, Niue, Philippines, Samoa, Tonga etc. [5]. In other parts of the world only periodic forms of mf of *W. bancrofti* have been observed. De Meillon and Sebastian (1967b) [6] studied the microfilarial periodicity in humans in Rangoon, Burma. They reported that the density rises sharply after sunset and then is maintained at a higher, even if variable, level until just before sunrise. Gatika et al (1994) [7] observed the microfilariae periodic pattern of *W. bancrofti* in Kenya. The peak time was 00.56 and the period index was 117.08, confirming nocturnal periodicity. Simonsen et al (1997) [8] reported the nocturnal periodic pattern with a peak at 01:52 in Tanzania. Weerasoorya et al (1998) [9] reported the peak in mf density at 01:11. In India, both periodic and sub periodic forms of *W. bancrofti* have been recorded. The sub periodic form of *W. bancrofti* has been reported from Nicobar island only. In India, nocturnal periodicity has been observed by Iyengar (1938) [10] and Raghavan & Krishnan (1949) [11], Srivastava and Prasad (1969) [12] studied the periodicity of *W. bancrofti* microfilariae at Lucknow. The mf showed nocturnal periodicity, emerging in large number as the hours of the night proceed, being the highest at midnight and then the number starts falling, disappearing in most of the cases by 08:00. Rajagopalan et al (1977c) [13] reported that except dusk and dawn, higher number of microfilariae was found in the blood at all hours of the night and no definite peak was observed. Tanaka (1981) [14] reported that in the nocturnally periodic type, the peak microfilariae density in the peripheral blood emerges between 22:00 and 02:00. Sinha (1975) [15] in Bhagalpur, Bihar, observed microfilariae in blood from 20:00 onwards, reaching a peak at 22:00. Vanamail and Ramaihia (1991) [16] reported that the peak biting activity of the vector and the peak appearance of mf in the peripheral blood synchronize with each other and the peak occurred at about 01:00, which accounted for the optimum infection of the vector population, in Pondicherry, Kumar et al (1995) [17] studied the peak prevalence of mf in three districts of Orissa. The peak prevalence of mf in Puri district occurred during 20:30-21:00; in Khurda district during 19:30-20:00; and in Nayagarh district the first peak occurred during 20:00-20:30 and a second peak beyond 21:30. They also reported low prevalence of microfilariae in winter season and higher prevalence in rainy season. Tewari et al (1995) [18] observed sub periodic mf with peak at 18:00 and a trough between 03:00-06:00. Chandra (1995) [19] reported highest peak in the third quadrant of night in both urban and rural areas of Calcutta.

The foregoing suggest: (a) that the emergence of microfilariae (*W. bancrofti*) in the peripheral circulation of infected humans exhibits a remarkable rhythm; (b) that this rhythm could be either nocturnal or diurnal or crepuscular (c) and that the pattern of the rhythm may vary as a function of geographical location. However, most of the studies performed earlier have not taken any cognizance of rhythms in mf appearance other than that in...
the domain of circadian frequency. Further, in the vast area of Chhattisgarh region similar studies are altogether lacking. Therefore, in this Section an attempt has been made to look into the rhythmic behavior of mf emergence of *W. bancrofti* in human subjects on 24-hour scales at Raipur. This study may highlight importance of multi frequency rhythms in mf appearance and may have a bearing on strategies aimed at prevention, cure and management of filariasis.

**Materials and methods**

**Collection of blood sample:**

Finger prick blood samples (20 µl) were collected from each microfilaraemic subjects. Two blood smears were prepared from each volunteer simultaneously. The blood slides were dried, dehaemoglobinized, stained with Giemsa and examined under the microscope. Number of microfilaria present in each slide was recorded. Human subjects were confirmed positive for microfilariae of *W. bancrofti* by routine blood smear examination.

**Study of circadian rhythm:**

For detecting circadian periodicity in microfilarial density, 6 microfilaraemic subjects, 4 males and 2 females, in the range of 6-35 year-old were selected. The blood samples were collected at 2-h intervals regularly over a 32-hours period.

![Circadian Rhythm of W. bancrofti mf in microfilaraemic blood](image)

**Results**

A statistically significant circadian variation in emergence of microfilariae in peripheral blood was validated in all microfilaraemic subjects (Table 1). At group level, when the data from 6 subjects were pooled, the curve clearly demonstrates a significant circadian rhythm in microfilariae density (Fig. 1). In all microfilaraemic subjects’ blood, the mf density found to increase from 20:00 and reached at peak on 02:00 hours. After that a decrease in mf density starts and found to be null on 08:00 and became zero on 12:00 no appearance of mf found till 04:00 to 06:00 hours.

**Subject wise analysis of circadian variation in mf density in peripheral blood:**

**Subject-1 (Code # DT-1)**

In the subject-1 the mean maximum density of mf found to be 170 on 02:00 hrs and mean minimum density was 4.5 on 08:00 hrs. The density was found to be zero between 12:00 to 18:00 hrs.

**Subject-2 (Code # DT-2)**

The mean maximum density of mf in this subject was found 155 on 02:00 hrs and mean minimum density was 1 on 16:00 and 18:00 hrs. The density was found to be zero on 12:00 hrs.

**Subject-3 (Code # DT-3)**

In this subject the mean maximum and minimum density of mf was found 145 and 0.5 on 00:00 and 16:00 hrs respectively. The density was zero on 12:00 hrs.

**Subject-4 (Code # DT-4)**

The mean maximum and minimum density of mf in this subject was 135 and 1 on 00:00 and 12:00 to16:00 hrs respectively. The density was found to be zero on 08:00 hrs.

**Subject-5 (Code # DT-5)**

In the subject-5 the mean maximum density of mf found to be 134 on 00:00 hrs and mean minimum density was 0.5 on 08:00 hrs. The density was not found to be zero elsewhere.

**Subject-6 (Code # DT-6)**

The mean maximum density of mf in this subject was found 20.5 on 20:00 to 22:00 hrs and mean minimum density was 0.5 on 08:00 hrs. The density was found to be zero on 12:00 hrs.
Discussion

Almost all living organisms, some prokaryotes and all eukaryotes from fungi to humans, exhibit rhythms in many of their vital, physiological and behavioral activities. The range of frequencies that has been found in the living system extends from cycle of less than a second to cycles of a year or more. In general, rhythms are endogenous in origin and represents adaptation to the geophysical periodicities those exist in nature. The term circadian is coined by Halberg [20,21] is used to describe any rhythmic phenomenon with a period near to 24 h. Intra-diurnal rhythms that has a period approximately equal to 24 hour. In contrast, infradian rhythms have period greater than or equal to 28 h. Thus, circannual rhythms having a period equal to 365.25 days could well be included in to the category of infradian rhythms [22], in the same token 7-day and 15-day bioperiodicities are also considered under infradian category. The present study was planned to investigate the circadian periodicities of mf in capillary blood of microfilaraemic subjects at Raipur.

The results of the present study suggest that the periodicity in microfilariae of *W. bancrofti* is nocturnal at Raipur city. However, the results of present investigation are not adequate to identify the cue precisely. Nevertheless, since microfilariae themselves are not directly exposed to photoperiod, one of the strongest entertainers, it is reasonable to think that the cue that provides temporal signals to the filarial worms could be internal with an origin in their human host. A possible candidate is “host body temperature”. It has been unequivocally established that humans possess an accurate biological clock for body temperature [23-26]. The time course in human body temperature reflects an acrophase (peak) in the afternoon and a bathyphase (trough) in the night [27]. Could it be that the mf have an ability to detect their hosts’ both temperature? Accordingly they set their own circadian rhythm in peripheral abundance in host’s circulation so that they are more in the periphery in the night when body temperature of the host is the lowest. Above presumption is speculative as in the present study body temperature of human subjects was not recorded. However, result of the experiments conducted by Hawking (1965) [28] on monkey support the above presumption. In his experiment when body temperature of monkeys was lowered in the day time the microfilaria count rose dramatically from 11 mf to about 55 mf per 20 mm³ blood.

Table 1. Circadian rhythm of *W. bancrofti* microfilariae in microfilaraemic patient’s blood.

<table>
<thead>
<tr>
<th>PATIENT ID</th>
<th>REPLICA TIES</th>
<th>MF COUNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAY FIRST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22:0 0 0</td>
</tr>
<tr>
<td>DT-1</td>
<td>R1</td>
<td>129 121 174 60 35 5 0 0 0</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>125 123 165 63 38 4 0 0 0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>127 122 170 62 37 5 0 0 0</td>
</tr>
<tr>
<td>DT-2</td>
<td>R1</td>
<td>125 134 157 87 67 2 0 1 1</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>133 141 153 85 52 2 0 1 1</td>
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<tr>
<td></td>
<td>M</td>
<td>129 138 155 86 60 2 0 1 1</td>
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<tr>
<td>DT-3</td>
<td>R1</td>
<td>108 146 153 136 142 4 0 1 7</td>
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<td></td>
<td>R2</td>
<td>104 144 133 127 144 12 0 0 0</td>
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<td>M</td>
<td>106 145 143 132 143 8 0 0.5 3.5</td>
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<td>DT-4</td>
<td>R1</td>
<td>11 24 28 16 6 0 0 0 1 0</td>
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<td></td>
<td>R2</td>
<td>13 22 30 16 8 0 0 0 1 2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>12 23 29 16 7 0 0 0 1 1</td>
</tr>
<tr>
<td>DT-5</td>
<td>R1</td>
<td>110 135 123 126 91 0 1 1 2</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>122 133 128 130 90 1 1 2 2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>116 134 126 128 91 1 1 1.5 2</td>
</tr>
<tr>
<td>DT-6</td>
<td>R1</td>
<td>3 8 7 5 4 1 0 2 0</td>
</tr>
<tr>
<td></td>
<td>R2</td>
<td>2 7 9 4 4 0 0 0 2</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>2.5 7.5 8 4.5 4 1 0 1 1</td>
</tr>
</tbody>
</table>
The reverse happened when the body temperature of monkeys was raised in the night. There are also many other factors, such as oxygen concentration in inspired air, increased CO$_2$, hyperventilation and exercise, those might have influenced the evolution of a circadian rhythm in mf density [29]. The present study clearly documents statistically significant high amplitude circadian rhythm in mf in human subjects regardless of gender, of Raipur district.

Notes and References

10. Iyengar, M.O.T.: Indian Medical Research Memoir, 30 (1938)1-179.
15. Sinha, V.P.: Rajendra Memorial Research Institute, (1975) 70-72.