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Microfilarial periodicity of *Wuchereria bancrofti* in Assam, Northeast India

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ABSTRACT

Background & objectives: Wuchereria bancrofti has shown nocturnal periodicity in India and other endemic countries of the world except pacific regions where non-periodic or diurnal sub-periodic forms have been reported. Presence of sub-periodic form of *W. bancrofti* in Andaman and Nicobar Islands, India and a case report of sub-periodic form of *W. bancrofti* from Mysore, India provide basis for exploring the periodicity pattern of microfilaria of *W. bancrofti* prevalent in Assam, Northeastern region of India. State of Assam has unique geographical location as its Northeastern region shares international boundaries with Nepal, China, Bhutan, Myanmar and Bangladesh. Evolutionary association of *W. bancrofti* found in Assam is not known and possibility of its link with *W. bancrofti* form, prevalent in neighbouring countries may not be ruled out. Hence, this study was undertaken to know the microfilarial periodicity of *W. bancrofti* in Assam.

Methods: Ten microfilaria positive adult male individuals having moderate to high microfilaraemia were selected. Informed written consent from each participant was obtained. The presence of microfilaria was observed at two hourly intervals over a period of 24 h using 50 µl of finger prick peripheral blood samples. Peripheral blood smears were processed, stained and examined under microscope and microfilaria counts were recorded.

Results: Data collected were calculated and analyzed using modified statistical method, and the periodicity curve was prepared. Typical nocturnal periodicity was observed at a peak time of 0003 hrs with a periodicity index of 136.2.

Interpretation & conclusion: Analysis of the data revealed nocturnal periodicity of the *W. bancrofti* prevalent in the Assam with peak periodicity about one hour ahead of the other states in India. Findings will be helpful in evaluation and monitoring of ongoing MDA programme for elimination of LF in Assam.

Key words Assam; circadian cycle; lymphatic filariasis; microfilarial periodicity; nocturnal periodicity; *Wuchereria bancrofti*

INTRODUCTION

Lymphatic filariasis (LF) caused by *Wuchereria bancrofti* is a neglected tropical disease and poses serious public health problem, affecting 120 million people living in 73 countries of the world[1]. One-third of the world's population infected with LF live in India and over 18 Indian states and the union territories are endemic for LF[2-3]. Approximately, 420 million people reside in endemic areas and 48.11 million are infected[2]. Bancroftian filariasis caused by *W. bancrofti* accounts for 95% of the total lymphatic filariasis cases in India[4]. The state of Assam has a large population of tea growing community especially in upper regions of Assam, where a large number of tea gardens are present. High prevalence of LF was recorded from various tea gardens of Assam in earlier studies[5-9]. The microfilariae of *W. bancrofti* may be found in the blood at all times, but its concentration in the peripheral blood of the infected humans follow a circadian periodic cycle which appears to be synchronized with the biting habits of the mosquito vectors[10-12].

Nocturnal periodic form of *W. bancrofti* is widely distributed in tropical and subtropical regions like Africa, Asia and Latin America while non-periodic or diurnal sub-periodic form is prevalent in the Islands of the South Pacific regions where maximum densities of microfilaria (mf) count is observed around 1630 hrs; and the distribution of sub-periodic form of *W. bancrofti* is limited to western Thailand where peak mf density in the peripheral human blood is observed at 2030 hrs[13-15]. In India also, sub-periodic form of *W. bancrofti* was recorded in Andaman and Nicobar Islands[16-18]. Bancroftian filariasis, in India is transmitted mainly by *Culex quinquefasciatus* which is a night biting mosquito and the mf periodicity is nocturnal except for those reported from Andaman and Nicobar Islands[16-19]. There is no report on microfilarial periodicity of *W. bancrofti* from Assam; therefore, the present study was undertaken in Dibrugarh district of Assam to observe the pattern of microfilarial periodicity.

MATERIAL & METHODS

Study location and collection of peripheral blood samples

The study was conducted in tea garden worker population of Dibrugarh district, Assam, Northeast India. It lies at a latitude 27°32'26.51"N and longitude 95°15'21.68" E with an altitude of 397 ft. The number of participants considered in the study was selected on the basis of available literature on earlier studies of microfilarial periodicity and in consultation with biostatistician[12, 20-22]. In total, 10 mf (*W. bancrofti*) carriers aged between 18 and 40 yr harbouring moderate to high density of mf in their peripheral blood were recruited after informed written consent. All the 10 participants were hospitalized in the hospital of the tea estate for a period of 30 h. A total of 50 µl of finger prick blood sample was taken from each individual on glass slide at two-hourly intervals (12 collections) using a micropipette. Sample collection started at 1830 hrs and the last collection was done at 1630 hrs on the following day (Table 1). Slides were processed and stained with Giemsa following standard protocol[23]. Slides were then examined under the microscope for quantification of mf density.

Analysis of data

The method of Sasa and Tanaka[24-25] based on goodness of fit to the harmonic wave equation was used for analysis of periodicity data. Assuming that the mf periodicity follows a 'harmonic wave type' the relationship between mf density (y) and hour of day (h) was expressed as:

$$y = m + a \cos 15(h - k)^\circ \quad \text{... (1)}$$

Where, m, a and *k* are the mean, amplitude and peak hour density. As 24 h correspond to 360°, the hours 0 to 24 are multiplied by 15 to correspond to angle 0° to 360°.

The harmonic equation of Sasa and Tanaka (1) was simplified by Aikat and Das[26] as follows (2) and was used in the present study for estimation of the parameters m, a and *k*.

$$y = m + b \cos 15\,h + c \sin 15\,h \quad \text{... (2)}$$

Solving equation (2) for *b* and *c* we have,

$$b = a \cos 15\,k \quad \text{... (3)}$$

$$c = a \sin 15\,k \quad \text{... (4)}$$

$$\text{Hence, } a^2 + b^2 = c^2 \text{ or } a \text{ (amplitude)} = \sqrt{b^2 + c^2} \quad \text{... (5)}$$

$$\tan 15\,k = c/b \quad \text{... (6)}$$

Thus, problem of estimation of m, a and *k* of (1) is reduced to that of measuring the parameters of (2) and then application of relationships (5) and (6).

Now, the least square estimates of *m*, *b* and *c* of equation (2) are as follows:

$$m(mean) = \frac{1}{n}\sum y \quad \text{... (7)}$$

$$b = \frac{2}{n}\sum y \cos 15\,h \quad \text{... (8)}$$

and

$$c = \frac{2}{n}\sum y \sin 15\,h \quad \text{... (9)}$$

Table 1. Observed individual microfilarial density, and the observed and theoretical mean mf ratios, at two-hourly intervals for the 10 study individuals

Individual No.	Age/Sex	Mf density (mf/50 µl) at the hours of examination (hrs)											
		1230	1430	1630	1830	2030	2230	0030	0230	0430	0630	0830	1030
1	20 M	0	0	0	32	180	317	439	188	211	33	0	0
2	40 M	1	2	6	225	354	395	565	401	304	78	4	2
3	18 M	0	0	0	33	144	114	136	152	54	83	0	0
4	18 M	0	2	0	85	138	90	156	141	87	91	1	0
5	20 M	1	0	1	10	103	85	106	57	43	43	0	0
6	35 M	1	2	16	95	82	186	72	76	96	8	3	1
7	22 M	0	1	0	4	25	19	23	19	13	11	0	0
8	28 M	0	2	4	117	163	186	197	143	104	3	0	0
9	25 M	0	1	7	58	177	222	276	164	141	16	6	4
10	24 M	0	0	0	5	28	40	49	27	32	23	0	0
Observed mean mf ratio (Y)		0	1.4	4.2	91.8	193.3	229.4	280.9	190.5	151.5	54.2	1.4	1.4
(% of Max)		(0)	(0.5)	(1.5)	(32.7)	(68.8)	(81.7)	(100)	(67.8)	(53.9)	(19.3)	(0.5)	(0.5)
Theoretical mf ratio (Y')		–35.2	–9.2	46.2	116	181.5	225.1	235.2	209.1	153.8	84	18.5	–25.1
(% of Max)		(–15)	(–3.9)	(19.6)	(49.3)	(77.2)	(95.7)	(100)	(88.9)	(65.4)	(35.7)	(7.9)	(–10.7)

Data were analysed keeping into account of mf ratio and not the actual mf count which eliminated the effect of individual differences in mf intensity as described by Sasa and Tanaka[24, 27]. The mean mf intensity for the 12 blood collections was calculated. The observed mf ratio Y was calculated for each hour as the percentage of the individual mean mf density for each examination hour divided by the mean mf count. Using the method of Aikat ad Das[26], the values of both a (amplitude) and m (mean) were estimated directly. The periodicity index was calculated as relative amplitude or coefficient of variation of observed mf count by, $(\frac{a}{m} \times 100)$ ... (10)

Ethical considerations

The study protocol was approved by the Institutional Ethics Committee (IEC) of Regional Medical Research Centre, Dibrugarh, Assam. Investigators explained the objectives of the study to each participant and written informed consent was obtained from each of them. The females were reluctant to participate, hence not included in this study.

RESULTS

Parasitological observation

Results of the two-hourly blood examination of 10 microfilaraemic individuals are shown in Table 1. The observed individual peak hours ranged from 2230 to 0230 hrs. The mean mf ratios for the 10 individuals at each examination time and the mean mf ratios expressed as percentages of the maximum value were calculated (Table 1). The relative periodicity (observed mf periodicity graph) curve (Fig. 1) was drawn by plotting the ratios obtained as the percentage of the individual mean mf density for each examination hour divided by the mean mf count (Y-values shown in Table 1). A clear nocturnal pattern of periodicity was observed, with a peak time around 0003 hrs.

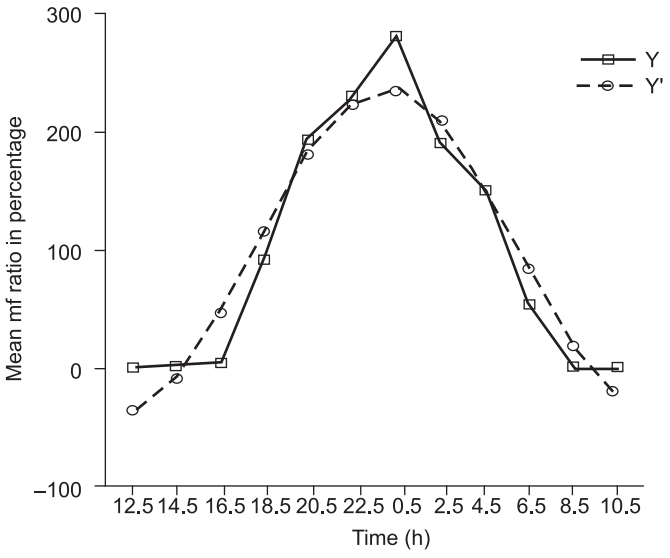

Fig. 1: The observed (Y) and Ë%, the theoretical (Y') *W. bancrofti* periodicity curves. The curves are based on the observed and theoretical mean mf ratios, respectively, expressed as percentage.

Statistical analysis

The statistical analysis of the periodicity data according to the method of Aikat and Das[26] is presented in Table 2. The analysis indicates a periodicity index of 136.2. In Table 2, 0.5 h corresponds to examination hour 0030, so 0.1 h will be equal to 0006 hrs. Therefore, the *k* value of 0.05 h will be equal to examination hour 0003. According to Sasa and Tanaka[24-25], D-values of 100, indicate that the *W. bancrofti* form involved are of the nocturnal periodicity. From the Y-values of Table 2, the theoretical relation between mf ratios (Y) and hour of the day (h) for the 12 examination times were calculated as per the theoretical mf periodicity equation

$$Y' = 100 + 103.9 \cos 15\,h + 54.9 \sin 15\,h$$

The theoretical mf ratios (Y' values) for the 12 examination hours, calculated by this equation, are presented in Table 1. Theoretical mf ratios were subsequently expressed as percentages and also as percentages of the maximum value as shown in Table 1. The theoretical mf ratios (Y' values) obtained as percentage of the individual theoretical mf ratios for each examination time were used

Table 2. Trigonometric analysis of the observed mf ratios, according to the method of Aikat and Das[26]

Examination (hrs)	Time (h)	Observed mean mf ratio (Y)	Y^2	Y cos 15 h	Y sin 15 h
1230	12.5	0	0	0	0
1430	14.5	1.4	1.9	–1.1	–0.9
1630	16.5	4.2	17.6	–1.6	–3.9
1830	18.5	91.8	8427.2	11.9	–91
2030	20.5	193.3	37364.9	117.7	–153.4
2230	22.5	229.4	52624.4	211.9	–87.8
0030	0.5	280.9	78904.8	278.5	36.7
0230	2.5	190.5	36290.3	151.1	115.9
0430	4.5	151.5	22952.3	57.9	139.9
0630	6.5	54.2	2937.6	–7.1	53.7
0830	8.5	1.4	1.9	–0.9	1.1
1030	10.5	1.4	1.9	–1.3	0.5
Total		1200	239524.8	817	10.8

to draw the theoretical mf periodicity curve shown in Fig. 1. It is obvious from the figure that the observed and theoretical mf periodicity curves follow each other closely, especially between 2030 and 0430 hrs, and the above equation, thus accurately expresses the relation between mf ratio and time of the day for the study area.

DISCUSSION

World Health Organization has set up a goal for elimination of LF by 2020. India has joined hands with WHO and put the year 2015 as the target year for elimination of LF from India. The main stay of the LF elimination programme is mass drug administration (MDA) which requires monitoring of mf status before each round of MDA through night blood surveys. Ideally, survey done during peak hours of mf density in the peripheral blood of mf carriers provide accurate results of mf status for assessing the effectiveness of MDA programme in a given location. Thus, the information about peak abundance of mf in the human peripheral blood is pertinent and hence, periodicity of mf is to be ascertained. Studies conducted on mf periodicity elsewhere has shown that there are many factors which influence circadian cycle of mf, but vector biting rhythm play an important role in periodicity pattern of mf[10-12] . In India, three genetically determined physiological races exist for *W. bancrofti* which are the nocturnally periodic, nocturnally sub-periodic and diurnally sub-periodic forms[28].

The state of Assam is located in the Northeastern part of India and is endemic for bancroftian filariasis. Northeastern region of India has unique geographical location and shares international boundaries with Nepal, China, Bhutan, Myanmar and Bangladesh. The evolutionary association of *W. bancrofti* circulating in Assam is unknown; however, there are reports of two distinct strains of *W. bancrofti* exhibiting two different microfilarial periodicity from another South Asian country, Thailand[29]. Further, one strain of *W. bancrofti* in Thailand is nocturnally sub-periodic while Myanmar strain is nocturnally periodic[30]. These evidences strengthen the view that presence of different strains of *W. bancrofti* in a close geographical area is possible.

In our study the peak hour of mf density in the peripheral blood (0003 hrs) was found somewhat coinciding with the peak biting hour of *Cx. quinquefasciatus* (from 2200 to 2300 hrs) reported in a study in India[31]. In India, *W. bancrofti* exhibit nocturnal periodicity and vector involved in transmission is *Cx. quinquefasciatus*; however, in Andaman and Nicobar Islands of India sub-periodic form of *W. bancrofti* has been reported and vector involved is *Ochlerotatus niveus* (day biter)[19]. Simonsen *et al*[21] while studying periodicity of *W. bancrofti* in Tanzania reported nocturnal periodicity and discussed importance of sampling time while dealing with epidemiological studies. In the present study, we also recorded presence of mf in the peripheral blood during day time, but at a very low level (0–16) which attained a peak density during night around 0003 hrs. This change in the density of mf was independent of the individual status of mf count of the participants. The similar findings were also observed by Fontes *et al*[22] while studying periodicity of *W. bancrofti* in western Brazil. It may be a limitation of our study that we did not recruit female participants as they were reluctant and could not consent for this study.

Our finding endorses the nocturnal form of periodicity of *W. bancrofti* prevailing in Assam and supports the view that most of the Indian forms of *W. bancrofti* is nocturnally periodic in nature. However, the peak mf count in Assam is about one hour ahead to that in other parts of the country which could be due to the fact that sunset time in Assam is one hour earlier than in western/southern/northern parts of the country. Our findings add a new data on mf periodicity from this region of the country. The outcome of this study is relevant in the context of ongoing MDA programme for elimination of LF from Assam where night blood survey is an important component of LF elimination.

Conflict of Interest

Authors have no conflict of interest with regard to this communication.

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