The dynamics of microfilaraemia and its relation with development of disease in periodic *Brugia malayi* infection in South India

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SUMMARY

Rates of acquisition and loss of *Brugia malayi* microfilaraemia were estimated using the parasitological data of a cohort of population in Shertallai, South India. The rate of acquisition of microfilaraemia was found to be dependent on age but not gender. The decline in the rate of acquisition of microfilaraemia in adults above 35 years could be due to the development of acquired immunity. The mean reproductive lifespan for the periodic *Brugia malayi* adult female worm was estimated to be 3·4 years and it was independent of host age and gender. The age-specific estimated proportion of population at risk (microfilaria carriers who lost their microfilaria in course of time) of developing lymphoedema approximately mirrored the observed age specific prevalence of lymphoedema in the study population. On an average, 99% of population at risk developed manifestations of disease. The estimated population at risk of developing disease in different endemic areas is compared and its epidemiological significance is discussed.

INTRODUCTION

Lymphatic filariasis caused by *Brugia malayi* is an important public health problem particularly in India and other South East Asian countries [1]. Many aspects of the transmission and the dynamics of microfilaraemia and disease in endemic populations remain poorly understood or quantified [1, 2]. Detailed studies on several epidemiological aspects and on the control of brugian filariasis have been initiated in Shertallai part of Kerala state, India [3–6]. In this communication we have used the reversible catalytic model [7–9] to describe the age specific estimates of the rates of gain and loss of *B. malayi* microfilaraemia based on longitudinal data. These estimations enable us to calculate the proportion of people who had been microfilaraemic but have subsequently become amicrofilaraemic. This population is assumed to be at the risk of developing disease manifestations as was observed in bancroftian filariasis [10]. This estimated proportion at risk in different age classes is compared with the observed age-prevalence of brugian filariasis disease in an endemic population. The same risk factor is calculated from cross-sectional data collected from other endemic areas for comparison.

MATERIALS AND METHODS

Conceptual frame work

The reversible catalytic models developed by Muench [7] were used in the earlier studies [8–10] to describe the transmission dynamics of *W. bancrofti*. The equilibrium dynamics of the microfilaria (mF)-negative (U) and mF-positive (I) portions of the human population were described by coupled differential equations:

$$dI/dt = -\mu I + \lambda U, \quad (1)$$

$$dU/dt = -\lambda U + \mu I, \quad (2)$$

where λ is the instantaneous rate of gain of microfilaraemia by an uninfected individual; and μ is the instantaneous rate of loss from an infected individual. The model assumptions were same as in our earlier study [9]. Since the peripheral blood smear examination fails to detect non-fecund pre-adults and single worm or single sex infections, the present analysis was restricted in calculating gain and loss only for fecund infections.

In an endemic area where transmission had been interrupted by several control measures [3], the instantaneous rate of loss of microfilaraemia μ and fecund life span ($1/\mu$) of adult parasite were estimated using the following method:

$$\mu = -\ln(I_t/I_0)/t,$$

where t is the time period between the estimation of I_0 and I_t . An estimate of the rate of acquisition of microfilaraemia by uninfected and susceptible populations was obtained by substituting the estimate of μ in the solution of equation (2):

$$U_t/U_0 = \exp[-(\lambda + \mu)t] + \mu/\lambda + \mu(1 - \exp[-(\lambda + \mu)t]).$$

In reality, the uninfected population is likely to include a proportion of individuals who have a history of microfilaraemia and become mF-negative as they have developed disease. As these individuals are assumed to be immune to further infections [11], the true susceptible population for infection was estimated using the relationship described earlier [9]. Based on the rate of acquisition and the rate of loss of microfilaraemia, the estimated mF prevalence was obtained as:

$$\text{est}_{\text{mf}} = (\lambda/\mu) 100. \quad (3)$$

Based on the similar catalytic models Hairston and Jackowski [8] suggested that the cumulative proposition of people at time t who had ever been mF-positive (I_t) could be estimated from:

$$I_t = 1 - \exp(-\lambda t), \quad (4)$$

where the rate of gain of microfilaraemia was assumed to be independent of age. If however, the rate of acquisition is age dependent, as observed for bancroftian filariasis [9] the relationship for age specific values of λ is given by:

$$I_{t_2} = 1 - (1 - I_{t_1}) \exp[-\lambda t_1(t_2 - t_1)], \quad (5)$$

where I_{t_2} is the proportion who have ever been mF positive at time t_2 ; I_{t_1} is the proportion at time t_1 ; and λt_1 is the rate of gain of microfilaraemia between t_1 and

t_2 . Thus the proportion of population (R_t) who had been microfilaraemic but have become amicrofilaraemic and are assumed to be at risk of progressing to disease is given by

$$R_t = I_t - \text{est}_{\text{mf}}. \quad (6)$$

Direct estimation of age-specific values of λ requires data on the mF status of large, age stratified cohort examined longitudinally. Age specific values were iteratively estimated from age-prevalence data using the solution of equation (1):

$$I_{t_2} = [\lambda_t/(\lambda_t + \mu)\{1 - \exp[-(\lambda_t + \mu)(t_2 - t_1)]\} + I_{t_1} \exp[-(\lambda_t + \mu)(t_2 - t_1)]], \quad (7)$$

where I_{t_2} and I_{t_1} are the observed prevalence at ages t_2 and t_1 , respectively. Thus data from a cohort study was utilized for estimation of the proportion at risk and it can be compared with observed age prevalence of disease. All these analyses assume an equilibrium state where the age distribution of infection and disease reflects stable transmission over a period equivalent to the age-range examined.

Data source

Shertallai taluk in Kerala state is a highly endemic area for brugian filariasis and in 1986, the Vector Control Research Centre (VCRC), Pondicherry launched a programme to interrupt the transmission by various control measures [3]. As part of this programme cross-sectional parasitological and clinical surveys [3-5] were undertaken to study the pre-control epidemiological situation. To study the rates of loss and acquisition of microfilaraemia, parasitological resurvey was carried out in 1989, 3 years after the introduction of control measures. The treatment status of mF carriers detected in 1986, was also recorded.

Thus age stratified cohort populations examined longitudinally for mF both in 1986 and 1989 formed the data base for estimation of rate of gain and loss of microfilaraemia. Data on age-prevalence of microfilaraemia and disease obtained in an earlier study in Shertallai [12], Vaikom [13] and Thailand [14] formed the database for estimation of rate of acquisition in an iterative manner.

RESULTS

A total of 2275 persons was examined longitudinally for mF both in 1986 and 1989; of these, 257 individuals were found to be microfilaraemic in 1986.

Rate of loss of microfilaraemia

Among the 257 mF carriers of 1986 re-examined in 1989, 214 were treated but 43 cases did not take any kind of chemotherapy. Twenty-five of these 43 were shown to be amicrofilaraemic in 1989, accounting for a natural loss of microfilaraemia of 0.5814. Under the assumption that in a population where transmission was interrupted by various control measures, the instantaneous rate of loss of microfilaraemia (μ) and fecundic life span of adult parasite were found to be 0.2903 and 3.44 years respectively. The rate of loss of microfilaraemia was independent of gender ($\chi^2 = 0.016$; $P = 0.899$). The logit regression of μ against host age indicated that the rate of loss of microfilaraemia is also independent of host age ($\chi^2 = 0.0150$; $P = 0.9025$).

Table 1. Age-specific rate of acquisition of infection (crude and corrected) with respect to disease status in amicrofilaraemic individuals

Age gp. (years)	mF -ve in 1986	Proportion gaining infection 1989	Disease status in mF -ve individuals in 1986		Corrected proportion gaining infection
			Sampled	Disease rate (proportion)	
0-9	314	0.0032	608	0.0066	0.00321
10-19	506	0.0099	791	0.0392	0.01028
20-29	304	0.0132	679	0.0781	0.01427
30-39	287	0.0139	547	0.1225	0.01588
> = 40	607	0.0033	1259	0.2240	0.00425
Total	2018	0.0079	3884	0.1125	0.00893

Fig. 1. Age-specific instantaneous rate of acquisition of infection. $\bigcirc$ — $\bigcirc$, Corrected rate; $\square$ — $\square$, crude rate.

Rate of acquisition of microfilaraemia

Sixteen of 2018 amicrofilaraemic individuals who were examined longitudinally in 1989 had become infected, a rate of gain of 0.79%. This rate was independent of gender ($\chi^2 = 1.05$; $p = 0.3052$). The age specific rate of gain of microfilaraemia is presented in Table 1. The age-specific instantaneous rate of acquisition (λ) was estimated by substituting the overall estimate of μ in the solution of equation (2) and depicted in Fig. 1. The polynomial regression of λ indicated that the rate of acquisition is dependent on host age ($F = 436$; D.F. = 2, 2; $P = 0.0023$).

Since the amicrofilaraemic population included a proportion of individuals who were microfilaraemic in the past but subsequently became mF negative during the progression to disease, these individuals were eliminated from the presumed uninfected population using the disease status among amicrofilaraemic persons as followed for *Wuchereria bancrofti* [9]. Thus by considering the actual uninfected population who were susceptible for infection the corrected rate of gain of microfilaraemia was estimated (Table 1). Considering the corrected rate of gain and the overall estimate of μ , the corrected instantaneous rate of acquisition λ was estimated and depicted in Fig. 1.

Fig. 2. Age-specific observed (□--□), estimated corrected (○—○) and estimated crude (△--△) mF prevalence.

Estimated point prevalence of microfilaraemia

The age-specific estimated mF prevalence (crude and corrected) was obtained by using the equation (3) and compared with that of observed mF prevalence [4] in Fig. 2.

Proportion of population (R_t) at risk of developing disease

Since the present analyses indicated that the rate of acquisition of microfilaraemia λ is age dependent, the cumulative proportion of people at time t who had ever been mF positive (I_t) was estimated by using the equation (5). Thus the age-specific proportion of population (R_t) who had been microfilaraemic but have become amicrofilaraemic and are assumed to be at risk of progressing to disease, was estimated by using the equation (6). The linear regression analysis indicated that the risk factor is age dependent ($F = 35.38$; $P = 0.0082$). As there was no significant difference in the age-specific prevalence of disease, as a whole as well as individual manifestations between the sexes [4, 5] the gender specific analyses were not carried out.

Probit regression analysis indicated that the observed age-specific prevalence of disease was linearly related to the estimate of age specific risk ($r = 0.9602$; $P < 0.05$). On an average, 99% of population at risk developed manifestations of disease. Similar analysis showed that the age-prevalence of filariasis disease was linearly related to the age-prevalence of lymphoedema ($r = 0.9728$; $P = 0.0054$). For all age classes, an average 97% of cases of diseased persons had lymphoedema. Multiplying the age-specific values of R_t by the conversion factor 0.9703 yielded an estimated age-prevalence for lymphoedema and compared with the observed values for the population in the study area (Fig. 3).

Lymphoedema was classified into recent oedema (grade I) and chronic oedema (grades II and III) and the details of these have been given earlier [5]. The age-specific prevalence of these two grades of oedema was compared with that of proportion at risk of developing lymphoedema (Fig. 4). Probit regression analysis indicated that on average 77 and 93% of population at risk developed recent

Fig. 3. Age-specific prevalence of lymphoedema (|—|) with 95% confidence limits and risk (○—○) of developing lymphoedema.

Fig. 4. Age-specific prevalence of lymphoedema in relation to grades (Δ — Δ , grade I; $\circ$ — $\circ$, grades II and III) and, risk ($\square$ — $\square$) of developing lymphoedema.

oedema and chronic oedema respectively. It was observed that while the chronic oedema prevalence showed a monotonic age-dependent rise, recent oedema was more or less uniform beyond 15 years of age.

Estimation of R_t based on age-prevalence data from other endemic areas

The present analyses were carried out by considering only the natural loss of microfilaraemia and it was assumed to be constant for the same parasite in the other endemic areas also. Thus by taking the overall estimate of μ the age-specific rate of acquisition of microfilaraemia λ was estimated in an iterative manner using the equation (7) and based on the age prevalence of microfilaraemia (Table 2) observed in earlier studies in Shertallai [12], Vaikom [13] and Thailand [14]. For these areas the age-specific estimated mF prevalence, the cumulative proportion of population who had ever been mF positive (I_t) and proportion of population (R_t) at risk of progressing to disease were estimated using the equations (3), (5) and (6) respectively.

Table 2. Age-specific mF prevalence (observed) in different endemic areas of *B. malayi*.

Age gp. (years)	Shertallai (1955)		Vaikom (1969)		Thailand (1953)	
	Sampled	mF rate(%)	Sampled	mF rate(%)	Sampled	mF rate(%)
1-5	819	13.5	42	2.4	221*	10.9
6-10	915	22.0	82	4.9	530	19.6
11-20	1739	22.4	215	9.3	1155	18.8
21-30	1977	23.5	212	8.0	765	21.0
31-40	1265	19.7	153	7.2	559	22.5
41-50	894	20.3	122	11.5	501	23.2
> 50	854	19.4	138	10.9	381	30.2
Total	8463	20.9	964	8.5	4112	21.0

* Sampled individuals in the age group 2-5 years only.

The age-specific estimated R_t based on the earlier study [12] in the same area was compared with the corresponding disease prevalence (Fig. 5a) which ranged from 2.7% (1-5 years) to 45.5% (> 50 years). The significant linear relationship ($r = 0.9808$; $P < 0.05$) between the age-specific risk factor and the observed disease prevalence showed that about 81% of population at risk developed disease manifestations. In Vaikom, the overall disease prevalence was 8.9% and it ranged from 0% (1-5 years) to 13.8% (> 50 years). The estimated R_t of this area was compared with the observed disease prevalence (Fig. 5b). In this area also, a significant correlation could be obtained between risk factor and disease prevalence ($r = 0.7833$; $P < 0.05$), however on average only 72% of population at risk developed disease. An average disease prevalence of 5.2% was observed in another area in Thailand, and it was minimal (0.0%) at 2-5 years and maximal (13.7%) above 60 years of age. The age specific estimated R_t for this area was compared with the observed disease prevalence (Fig. 5c). The age-specific risk factor was again linearly related ($r = 0.7481$; $P < 0.05$) with disease prevalence however, only 56% of population at risk developed disease. In all these areas the estimated age-specific R_t was at a higher level compared to the corresponding observed disease prevalence. For these surveys, the age specific pattern of lymphoedema and grades were not available.

DISCUSSION

In the present analyses, reversible catalytic models were used to study the dynamics of microfilaraemia and disease in periodic *B. malayi* infection and it was possible to estimate the natural rates of loss and acquisition of microfilaraemia as well as fecund life span of adult parasite. The accuracy in estimating the rate of loss of microfilaraemia μ is subjected to various factors. The current estimate of μ was based on the assumptions that conversion of positive to negative takes place at a constant rate and the individuals who loose microfilaraemia do not regain it (at least during the 3-year period of observation). Though the validity of the assumption was not tested directly, post control evaluations have shown that infectivity rate in vector population was zero (unpublished observations), suggesting that the possibility of reinfection was negligible. The rate of loss of

Fig. 5. Age-specific disease prevalence (|—|) with 95% confidence limits and risk (○---○) of developing disease in Shertallai during (a) 1955, (b) Vaikom during 1969, and (c) Thailand during 1953.

microfilaraemia was found to be independent of host age indicating that the fecundity or death of adult female worm is independent on host age as was also observed in periodic *W. bancrofti* infection [9]. The present estimation of the fecund life span of *B. malayi* is close to the previous estimates of 3–4 years which was based on long-term observations on subperiodic *B. malayi* [15], but shorter than that of *W. bancrofti* infection (5.4 years) estimated in Pondicherry [9]. The decline in the rate of acquisition of *B. malayi* microfilaraemia in the age group

above 35 years is more marked when compared to the fall in the rate of acquisition of *W. bancrofti* microfilaraemia [9] in the same age class and this could probably be due to the variations in host parasite interaction between the two species. The age-specific predicted prevalence of mF based on corrected rate of acquisition and the rate of loss of microfilaraemia closely mirrored the observed prevalence. The general decline in the predicted values may either be due to a higher rate of loss of microfilaraemia or the decline in the rate of acquisition. The variation between the observed and predicted mF prevalence is more marked in the older age classes and this may be due to immune responses and the development of clinical manifestations (disease individuals are mostly amicrofilaraemic). The present analyses do not pose any bias in the technique adopted, but the decline in the rate of acquisition showed in the present study confirms earlier reports on the decline in the occurrence of newer infections in this locality [12, 16, 17]. The reasons for such a natural decline has been attributed to the replacement of *Pistia stratiotes*, the most preferred host plant of vector mosquitoes by *Salvinia molesta* [18] and several other factors in combination including vector control measures [4].

The age-specific predicted proportion of population at risk of developing lymphoedema was observed to be within the confidence limits of lymphoedema prevalence except in the age groups 20–40 years for *B. malayi* infection. These findings are in agreement with the hypothesis that mF are produced following the infection and destroyed by the host immune response and a proportion of them develop clinical symptoms [19]. However, in older age classes (20–40 years) the proportion predicted to be at risk was more than the disease prevalence. This overestimate may either be due to disappearance of acute symptoms spontaneously or the long duration required to develop chronic manifestations [20]. As reported earlier [11] the variation in responsiveness to the parasite that results from specific immune responses as well as immunosuppression among population may also be one of the causes for the over estimation of risk factor. The risk factor was found to be overestimated when compared with observed prevalence of recent oedema whereas it was close to the observed prevalence of persistent oedema. This difference could be due to the duration of oedema. Lymphoedema cases with less than 6 months duration are diagnosed as recent and all the lymphoedema cases beyond 6 months as persistent oedema and due to such cumulation the prevalence of persistent oedema cases record higher than recent oedema cases. The risk factor in *B. malayi* was found to be overestimated when compared to the disease prevalence whereas in *W. bancrofti* it was reported to be closely mirrored with disease prevalence [10]. Slow disease conversion [21] and absence of hydrocele cases in *B. malayi* may be responsible for the overestimation of risk factor.

Since the observed mF and disease prevalence in this area was reported to be above 20% in 1955 [12], 81% of risk population developed clinical manifestations. This indicates that a large proportion of risk population develop fresh clinical manifestations. Both mF and disease prevalence were almost equal which indicates that the introduction of infection was relatively recent which was also reflected from the low proportion of risk population developing disease (72%). The proportion of risk (0.56) developed clinical manifestations in Thailand was relatively lower than that of risk (0.77) developed into recent oedema in the present study. This could suggest that most clinical cases in Thailand could have

been of grade I nature and hence the disease prevalence in that area was lower than the risk of population suggesting again recent introduction of filariasis in that area at the time of the survey. There may be several other reasons also for the discrepancies observed in all these endemic areas.

(i) The status of infection (recent or old) is not known at the time of surveys in these areas. The disease prevalence was much higher (9.85%) compared to mF prevalence (2.31%) in the present study whereas in Vaikom both disease and microfilaraemia were nearly equal (disease: 8.9%; mF: 8.5%). In Thailand on the other hand, the mF prevalence was higher (21.0%) compared to disease prevalence (5.2%). These data could suggest that the three areas were at different levels of microfilaraemia and disease.

(ii) It is not clear whether the method of clinical examination and defining disease itself are uniform in different surveys (the details for earlier surveys are not available). Difficulties in comparing clinical survey data for bancroftian filariasis from different areas are well known [22].

(iii) The possible role of immune mechanism in the dynamics of microfilaraemia and disease may be different in different geographical areas in relation to the duration of exposure to infection.

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