EOSINOPHILIA, ANEMIA AND PARASITISM IN A RURAL REGION OF NORTHWEST THAILAND

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Abstract. Parasitic infections are prevalent in Thailand. The parasites' relationship with anemia and eosinophilia has been rarely reported due to limited health care access, especially in rural areas. We studied the prevalence and relationship between eosinophilia, anemia and parasitism in 169 Thai-Karens from Mae Lamung and Mae Chan subdistricts, Umphang district, Tak Province, using an automated complete blood counter, and microscopic examination for intestinal parasites and microfilaria. In Mae Chan, 5 individuals were microfilaremic, 72% of individuals examined were infected with at least one kind of intestinal parasites, and 50% were anemic, with normal mean red cell volume (MCV). In Mae Lamung, 46 % were parasitized (Triteeraprapab *et al,* 1997) but none were microfilaremic or anemic. In both populations, eosinophilia was prevalent (77 %). Hookworm infection was found to be significantly associated with eosinophilia, but not anemia nor microcytosis of red cells.

INTRODUCTION

There are a few regions of Thailand that are relatively inaccessible to health care facilities. Umphang district, Tak Province, located at Thai-Myanmar border, northwest of Thailand is in the mountainous area and is about 700-1,300 meters above mean sea level. Most of population are Karen with very low migration rate. Their health status is relatively unknown.

Tak Province is one of the 5 provinces that still has cases of lymphatic filariasis due to *Wuchereria bancrofti,* nocturnal subperiodic type, with the prevalence of 113 per 100,000 population (Filariasis Division, 1996). Umphang district consists of 6 subdistricts; Mae Lamung, Mae Chan, Mae Glong, Umphang, Mogro, and Nong Luang. Mae Lamung subdistrict has 1.75% in fected cases and Mae Chan has as many as 2.92% in one village. The residents have received mass treatment with diethylcarbamazine (DEC) twice a year according to national policy. (Filariasis Division, 1996).

Apart from filaria and malaria, little is known about the general health data of people in the remote areas. Infections with parasitic helminths are associated with malabsorption and malnutrition of hosts (reviewed in Eve *et al,* 1998). Complete blood count (CBC) is one of the most useful health screening laboratory tests. CBC is the gold standard in detection of anemia and red cell indices can suggest its causes. Therefore, as a part of a field survey for lymphatic filariasis in Umphang, Tak Province, we performed complete blood count in conjunction with identification of intestinal parasites, and microfilariae in the peripheral nocturnal blood. Mae Lamung and Mae Chan districts were selected for our study in concurrence with the survey of lymphatic filariasis. We found that while there were microfilaremia found in only 5 individuals, eosinophilia and anemia were found at an unexpectedly high prevalence together with parasitism in the same population.

POPULATION AND METHODS

Study area and population

Umphang district is located 250 km from the center of Tak Province, northwest of Bangkok, close to the Thai-Myanmar border. Umphang district is accessible by car, although it is a 6-hour drive from Mae Sot, a district where the Vector-Borne Diseases Center 18 located, via tortuous secondary road. Our study areas included Mae Lamung and Mae Chan subdistricts of the Umphang district. Most of the residents are field workers and seek health care only when they feel sick.

In cooperation with the local health workers and health officers from the Ministry of Public Health, 230 villagers were recruited for this study. All individuals who stayed home at the time we visited (20.00-02.00 hours) were included in the study.

Because the routine survey for lymphatic filariasis will cover only individuals staying at home during the visit at night while the remaining residents stayed at their agriculture field far away, it is difficult to obtain the real prevalence of the disease. During our visits, we stayed 3 nights in each village in order to maximize the number of participants. Most residents of villages are Thai-Karen. They were willing to cooperate and were well informed about the hazards of lymphatic filariasis as well as other parasitic infections. Verbal informed consent was obtained from each individual or each child's parent or guardian. All individuals who were sick were referred to hospital for proper management.

Complete blood count

Complete blood count was performed using Coulter MAXM (Mae Lamung samples) or Technicon H*3 automated electronic cell counter (Mae Chan samples). Hemoglobin (Hb) was measured in g/dl, red blood cell count in cells per microliter, and mean cell volume (MCV) in femtoliter (fl); while hematocrit (Hct), mean red cell hemoglobin concentration (MCHC) were calculated values from Hb, MCV and red blood cell count of each individual. White cell count and platelet count were reported in the same fashion. Differential count was confirmed by visual examination of blood smear. Absolute neutrophil count and absolute eosinophil count were calculated from the differential count and white blood cell count from each individual.

Stool examinations and blood films for microfilariae

Stool examination for parasites was performed by formalin-ether concentration technic previously reported (Triteeraprapab *et al,* 1997). Examination for filarial parasites was performed by thin and thick blood smear as previously described (Triteeraprapab and Songtrus, 1998).

Data analysis

All data were statistically analysed and plotted by program Excel 6.0. Correlation between stool parasite data and hematology data analysed by chi-square tests. Difference in hematology values between Mae Chan and Mae Lamung were analyzed by unpaired Student's *t*-test.

RESULTS

Population characteristics

Out of 230 individuals who were examined for microfilaremia from finger-prick blood, 5 were microfilaremic. Complete blood count was successful in 169 individuals, 121 adults (67 males and 54 females) and 48 children (Table 1). We could not obtain blood samples from some individuals because they were away from home to work in the field. The rest of blood samples were excluded because of technical failure (*eg* clotted and hemolysed samples). The majority of the studied population (73 %) were between 10-40 years. The studied population classified by age are shown on Table 1.

Table 1

Population characteristics stratified by age group.

Age group (years)	Male	Female	Total samples
<10	11	11	22
10-20	33	26	59
20-30	23	16	39
30-40	14	13	27
40-50	8	3	11
50-60	5	3	8
>60	0	3	3
Total	94	75	169

Complete blood count

There were some differences in the CBC findings between the two subdistricts. The mean hemoglobin level in males and females from Mae Lamung were 16.1 and 14.2 g/dl respectively, while the mean values from Mae Chan were significantly lower; 13.9 g/dl in males and 12.6 g/dl in females ($p < 2x10^{-5}$) (Table 2). The mean hemoglobin level in children from Mae Lamung was 13.5 g/dl. While none of the individuals from Mae Lamung were anemic, 50% of male and 29% of female adults

from Mae Chan were in the anemic range (Hb<14 g/dl in male or <12 g/dl in female). The red blood cell count and hematocrit were also significantly lower in Mae Chan villagers than in Mae Lamung.

Despite the difference in hemoglobin levels, both populations had similar mean red cell volumes (MCV, mean 96-97 fl, Table 2). Females had larger MCV (mean 99 fl) than males and their value was higher than the normal range. There was no correlation between hemoglobin level and MCV (data not shown), even in anemic subjects. There was slight difference in calculated mean red cell hemoglobin (MCH) and mean red cell hemoglobin concentration (MCHC) between the two populations.

The platelet counts were similar in both groups and not different from other populations (Athens, 1993). The mean total white blood cell count was higher in the Mae Lamung group (9,200/µl for males; 10,400 /µl for females, Table 2) than in the Mae Chan group (7,100/µl for males; 7,700 /µl for females). The difference was related to a lower absolute neutrophil count (ANC) in the latter group. The proportions of lymphocytes and eosinophils were similar between the two groups.

High prevalence of eosinophilia

Because of a strikingly high mean absolute eosinophil count (AEC) and wide deviation, the AEC from the population was plotted on a histogram (Fig 1). The majority of population (77.5%) from both subdistricts had eosinophilia, which was defined by AEC greater than 500/µl (Spry, 1988). Forty percent of individuals had AEC greater than 1,000/µl, and 11% had AEC greater than 2,000/µl. Therefore the prevalence of eosinophilia was very

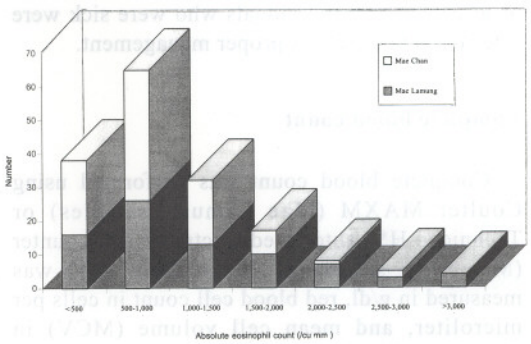

Fig 1–Distribution of absolute eosinophil counts (AEC) in the subjects of both subdistricts of Umphang.

Table 2

The complete blood count values of adults and children of two subdistricts in Umphang, expressed in mean ± standard deviation.

Age and sex	Adult Male		Adult Female		Children age<15 years	
	Mae Lamung	Mae Chan	Mae Lamung	Mae Chan	Mae Lamung	Mae Chan
Number of subjects	15	52	23	31	40	8
Red cell count (x106/µl)	5.5±0.6	5.0±0.6	4.8±0.4	4.4±0.7	4.9±1.0	7.3±2.1
Hemoglobin (g/dl)	16.1±1.1	13.9±1.2	14.2±1.1	12.6±1.3	13.5±2.5	14.5±1.3
Hematocrit (%)	52.5±2.8	47.9±3.9	47.7±4.0	42.9±5.5	45.3±8.1	49.7±4.2
Mean red cell volume (fl)	96.2± 8.0	97.0±8.3	99.5±7.8	98.8±6.9	93.0±6.6	100.9±8.5
Mean red cell hemoglobin concentration (g/dl)	30.8±1.3	29.1±1.8	29.8±1.1	29.6±4.6	29.8±1.5	29.1±1.1
Platelet count(x106/µl)	307.4±76.7	258.9±56.2	310.3±68.9	287.0±69.1	417.5±151.8	261.3±88.0
White blood cell count (x103/µl)	9.2±1.1	7.1±1.9	10.4±2.7	7.7±2.1	11.5±4.5	7.4±2.1
Neutrophil (%)	46.5±7.8	44.3±13.3	53.0± 9.1	43.3±8.2	42.0±11.3	51.3±8.8
Absolute neutrophil count (/µl)	4,299±966	3,176±1,295	5,604±2,175	3,434±1,407	4,955±2,163	3,664±719
Lymphocyte (%)	36.5±7.4	32.2±11.2	30.7±7.4	31.6±7.3	35.6±9.5	27.1±8.8
Monocyte (%)	4.7±1.1	9.8±5.5	5.2±1.0	9.0±3.1	4.6±1.2	10.2±4.1
Eosinophils (%)	9.3±6.0	10.9±6.4	8.5±5.2	13.3±5.7	12.2±7.4	8.1±5.8
Absolute eosinophil count (/µl)	857±554	778±511	882±576	1,015±514	1,521±1,206	633±663
Basophils (%)	1.1±0.2	0.4±0.4	0.9±0.3	0.5±0.4	1.1±0.5	0.8±0.4

high in this population.

For comparison, we also assessed eosinophil counts from 239 normal Thai subjects residing in Ratchaburi province whose blood samples were sent to our laboratory as a part of screening for thalassemia project with the same automated analyser (H*3). Eighty-two percent of this reference subjects had AEC < 500 /µl, while only 2.5% had AEC greater than 1,000/µl, and 0.8% had AEC greater than 2,000/µl. Therefore, if we chose AEC >1,000/µl as our cut-off point for eosinophilia in the Thai population, then at least 40% of Umphang subjects had eosinophilia.

Parasitism in Mae Chan subdistrict

Umphang is a known endemic area of lymphatic filariasis caused by *W. bancrofti*. In the Mae Lamung survey, none of the population examined were infected, however, five individuals were positive for microfilariae in villages 5 and 7 of Mae Chan district. All of the cases were asymptomatic.

Stool examination of those living in Mae Lamung was previously reported (Triteeraprapab *et al,* 1997). Of 112 individuals, 51 (41%) cases were found to harbor intestinal parasites. The most common parasites were *Ascaris lumbricoides* (49%), hookworm (25%) and *Trichuris trichiura* (22%). In this study, of 61 Mae Chan residents who provided specimens, 44 (72%) were positive for one or more kinds of intestinal parasites. The majority of parasite infections found were hookworm (36 cases = 59%), *Ascaris lumbricoides* (10 cases = 16%), *Opisthorchis* (5 cases = 8%), *Trichuris trichiura* (2 cases), *Taenia* (1 case), and *Giardia intestinalis* (1 case). Non-pathogenic parasites, *Endolimax nana, Entamoeba coli*, were found in 9 and 3 cases respectively. Thus, a significant number of population was infected with more than one intestinal parasites.

Hookworm infection

Infection by certain intestinal parasites, particularly hookworm, causes eosinophilia. Hookworm also causes iron deficiency and leads to anemia due to chronic blood losses (Crompton and Stephenson, 1990). Since hookworm infection was the most prevalent parasitic infection in Mae Chan subdistrict and had the infected cases enough to be analysed, we tested the association between hookworm infection and eosinophil count (AEC) and various red cell indices.

Of 51 Mae Chan villagers who provided both blood and stool samples, 28 of 40 people with eosinophilia were found to have hookworm infection while only 4 of 11 people with normal eosinophil count had hookworm infection (Table 3). This was found to be statistically significant by chi-square test whether AEC >500 or >1,000 /µl was used as a cut-off point. In contrast, none of the red cell

Table 3

Association between blood count values and hookworm infection in Mae Chan village subjects.

Hookworm infection	Not infested	Infested	Chi-square test
Number of subjects	19	32	p value
Red cell count <4.8 x10^6/µl	12	16	NS (p=0.36)
Hemoglobin <14 g/dl	7	16	NS (p=0.36)
Hematocrit <45 %	5	13	NS (p=0.30)
Mean red cell volume < 90 fl	3	13	NS (p=0.06)
Mean red cell hemoglobin concentration <28 g/dl	3	9	NS (p=0.32)
Platelet count >320x10^6/µl)	7	7	NS (p=0.25)
White blood cell count >7x10^3/µl)	10	21	NS (p=0.36)
Absolute neutrophil count >2,500/µl)	12	24	NS (p=0.37)
Absolute eosinophil count >500 /µl	12	28	p = 0.041
Absolute eosinophil count >1,000 /µl	3	14	p = 0.041

indices was significantly associated with hookworm infection. The degree of anemia as expressed by hemoglobin level seems to correlate with presence of hookworm infection, but it did not reach statistical significance (Table 3). There were no correlation between red cell indices and eosinophilia (data not shown). Thus from this study, we can confirm the relationship between hookworm infection and eosinophilia but not with anemia.

DISCUSSIONS

Baseline health survey data from the Thai-Karen population has seldom been reported. Together with the parasitic study, this was the first survey of hematological values in this population during the survey for lymphatic filariasis in that part of Thailand. The difficulty in obtaining specimens and labor-intensive nature of manual complete blood count in the past precluded the intensive survey in the remote areas. Using an automated CBC analyzer and better transportation system, we were able to report the baseline data of hematological profile from this Thai-Karen sub-population of Thailand.

In Thailand, a survey in 1996 showed that the prevalence rates of intestinal helminths decreased from 41.7% in 1991 to 35% in 1996 (Jongsuksantikul 1997). Such a high infection rate is considered a public health problem. We found that over 46% of people in Mae Lamung and 72% of Mae Chan villagers harbored at least one kind of parasite. This figure is much higher than the national average (35%).

When stratified by sex and age, the red blood cell count, hemoglobin, hematocrit, and calculated MCHC of Mae Lamung residents were not different from other populations reported. Although the hematology values in children varies with age, there were too few children in this survey to make a meaningful conclusion. The MCV in adults studied was higher than other population. The explanation of higher red cell volume has not been investigated. We suspect that nutritional deficiency may be an important factor. This village was below the poverty line and had difficulty to access for public transportation or health care.

Light infections of hookworm usually produce no recognized symptoms. The most prominent characteristics of moderate or heavy chronic hookworm infection is a progressive, secondary, microcytic, hypochromic anemia of the iron-deficiency type (Brown and Neva, 1983), due to the continuous loss of blood. Mae Chan residents had high prevalence of anemia (50%) as well as high prevalence of parasitic infections, particularly hookworm infection (59%), while Mae Lamung subjects had anemia and 25% had hookworm infection. There was a higher proportion of low MCV in the hookworm infected group than in the uninfected group (Table 3), but it did not reach a statistical significance. In addition, unlike iron-deficiency anemia typically seen with hookworm infection, the MCV of most anemic subjects were not uniformly low, and, in some cases, their MCV were high (data not shown). Thus the causes of anemia in this population remain to be investigated. It is possible that there are other nutritional deficiency anemias, such as that due to vitamin B12 or folate deficiency which are known to cause macrocytic anemia, with or without combined iron deficiency. It is also possible that there are peculiar forms of hemoglobinopathy in this ethnic group. Thus, further population survey of this region should include nutritional assessment of vitamin B12, folate, and iron status.

The most striking findings in the CBC was the high prevalence of eosinophilia in the peripheral blood in a majority of the population. Eosinophil counts can be reliably assessed by the automated analyzer (Krause, 1994). In most populations, eosinophil counts in healthy subjects are found to be less than 500 cells/μl ($<0.5 \times 10^9$ /l) (Spry, 1988; Gulati and Hyun, 1994). The eosinophil counts in Thai subjects were found to be higher than the above figure and was postulated to be related to parasitic infections (Hathirat *et al,* 1976) but the cut-off point to call eosinophilia was not proposed. Using another Thai population as reference the third percentile of the absolute eosinophil count was found to be 1,000 eosinophils per microliter. Therefore, even if we use this stringent criteria, at least 40% of the surveyed Thai-Karen still had eosinophilia.

Eosinophils are a striking feature of many parasitic diseases. Helminthic infections are the most common parasitic diseases that produce eosinophilia (Spry, 1988). Nematode infections account for the majority of patients with eosinophilia in tropical countries, especially in areas where filariasis, ascariasis and hookworm infection are endemic

(Spry, 1988). We could detect an association between eosinophilia and hookworm infection in Mae Chan subjects. This was consistent with the previous observation that hookworm causes eosinophilia (Maxwell *et al,* 1987; Ottesen, 1990). The prevalence of other parasites was too low to make a meaningful association. We did not analyze the Mae Lamung data because of the limited number of subjects who provided both stool and blood specimens. This difficulty in obtaining a paired specimen is an inherent problem in a field survey of this nature in the remote areas of Thailand.

Although hookworm was a ready explanation for eosinophilia, there were a number of eosinophilic people who tested negative for hookworm. This may be due to the limited in sensitivity of stool examination (Triteeraprapab *et al,* 1997). Umphang has been an endemic area for filariasis caused by *Wuchereria bancrofti*. The mass treatment with diethylcarbamazine through the filariasis control program by the Filariasis Division, Ministry of Public Health, has resulted in markedly reduced prevalence of this disease. None of the subjects from Mae Lamung but 5 from Mae Chan showed evidence of filarial infection in this survey.

Routine microscopic examination for microfilariae from nocturnal blood has low sensitivity (Triteeraprapab, 1997). Furthermore, repeated stool examinations are recommended for the diagnosis of parasitic infections. It is advisable for patients in endemic areas of filarial and other parasitic infections, especially ascariasis and hookworm infections, to take anti-parasite treatment once or twice a year (Spry, 1988). Though we presumed that the high prevalence of eosinophilic counts in this Thai-Karen population was mostly due to the parasitic infections, we should also beware of other diseases associated with eosinophilia. Furthermore, the high prevalence of anemia in this population warrant further investigation to define its causes.

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