ANEMIA, MALARIA AND HOOKWORM INFECTIONS IN A VIETNAMESE ETHNIC MINORITY

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Abstract. The aim of this study was to determine the prevalence of anemia and evaluate the relationship of malaria and helminth infections on anemia status in Phan Tien village, a mountainous ethnic minority community in southern Vietnam. This longitudinal study was performed from April 1997 to 2000 by measuring the hemoglobin concentration of 2,767 people who participated in six annual surveys at the end of the rainy seasons. Ferritin concentration was measured in 2000 to evaluate the proportion of iron deficiency anemia. The relation between malaria and intestinal helminth infections with anemia was investigated. Anemia was always over 43% and mainly associated with iron deficiency (80.1%). Using generalized estimating equations, a small but significant decline of the anemia prevalence was detected (OR: 0.805; p<0.0001). Malaria was significantly associated with anemia (OR: 2.408; p = 0.0006). There was no significant effect of the control of intestinal helminth infections on the time course of anemia (95% CI: -0.1548 to 0.1651).

INTRODUCTION

Anemia continues to be a major health problem worldwide. According to estimates of the World Health Organization (WHO), 2 billion people suffer from anemia in the world (Underwood, 1996). Anemia can lead to absenteeism, diminished learning ability, increased susceptibility to infection, growth retardation in children, reduced work performance, and increased accident rates (Basta *et al*, 1979; Dallman *et al*, 1980; Pollitt *et al*, 1985; Jansson *et al*, 1986; Walter *et al*, 1989; Stoltzfus *et al*, 2001). The causes of anemia are multifactorial. Micronutrient deficiencies such as iron, folate, and vitamin B12 are important causes of anemia (Provan and Weatherall, 2000). Iron deficiency (ID) is the commonest cause of nutritional anemia and is accountable for almost a million deaths annually (Hercberg and Galan, 1992; Cook *et al*, 1994). Sickle cell disease, other hemoglobinopathies, and thalassemia also contribute to anemia (Koerper *et al*, 1976). Anemia is often associated with parasitic diseases such as malaria and hookworm infections (Premji *et al*, 1995; Hopkins *et al*, 1997; Guyatt, 2000; Verhoef *et al*, 2002). Hookworm infection may cause anemia because it induces iron deficiency by chronic intestinal blood loss. The association between chronic anemia and malaria in the highly endemic regions such as sub-Saharan Africa and Papua, or with hookworm infections, may be so strong that anemia is often taken as a proxy indicator of malaria or hookworm control programs. However, in other regions, this relation is not so evident and other factors such as malnutrition or genetic factors may play significant roles.

To assess the rate of anemia in an ethnic minority population in Vietnam and to investigate whether malaria control and the control of hookworm infections may decrease the rate of anemia, we followed the population of Phan Tien, an ethnic minority commune in southern Vietnam, as a cohort, from before, until after, the control of malaria and hookworm infections.

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MATERIALS AND METHODS

Study site and population

The study was conducted in Phan Tien village, an ethnic minority commune located in a mountainous area of Binh Thuan Province, southern Vietnam. Hidden in the forest, it took several

hours to reach the village from the main road, even in the dry season, and it could not be reached by car in the rainy season. Before July 1994, there was no health care facility, and the water supply was from a small river next to the village. Electricity was introduced at the end of 2000.

The population of Phan Tien is composed of several ethnic minority groups. The number of individuals rose from 716 to 1,088 in 2000 due to immigration of settlers of different ethnic minority groups and a high birth rate (3.4% per year). People lived family wise with 5 to 6 persons in one house, with clay walls and a thatched roof. They did not have knowledge of personal hygiene, were often half-naked and walked barefoot especially infants and young children. People shared their domestic areas with animals. Human and animal excrement was disseminated around the village. People and cattle shared a small river as their water source for washing, and agricultural irrigation.

The economy of Phan Tien was and is still based on subsistence agriculture, mainly rice, and on what the surrounding forests have to offer. In general, the local economy and living standards, including sanitation, were very poor. Nutritional intake was marginal with low protein and micronutrient intake (Hung *et al*, 2002).

Demographic surveillance

A full census of Phan Tien was performed in 1994. Houses were numbered, and all individuals were registered. A record was completed for each individual with name, unique identifier, age, sex, household and ethnic group. Over the period of 1994-2000, surveys were routinely conducted at the end of the rainy season and a single additional survey was conducted at the end of the dry season in April 1997. During the surveys, demographic data were updated, including registering neonates, deaths, and population movement.

Measurements

Malaria was investigated during all surveys by on-the-spot microscopic examination of Giemsa stained thick- and thin-blood smears by experienced microscopists. The results were presented as positive/negative and specified by *Plasmodium* species. Intestinal helminth infections were only investigated in children less than 17 years old, starting in the survey of April 1997 and during the end of the rainy season from 1997 to 1999, as described elsewhere (Hung *et al,* 2005). Fresh stool samples were immediately examined by the Willis and Kato-Katz thick smear techniques and the agar plate technique for strongyloidiasis (Chaves *et al*, 1979; Arakaki *et al*, 1990; Nunez-Fernandez *et al*, 1991). Intestinal helminth infections and helminth species were presented as positive or negative.

Hemoglobin concentrations (Hb) were measured in all surveys from 1996 to 2000. Blood was collected by finger puncture for measurement of Hb with a portable hemoglobinometer (HemoCue®, AB, Angelhom, Sweden) (Hudson-Thomas *et al*, 1994; Sari *et al*, 2001; WHO/UNICEF/UNU, 2001).

Anemia was defined per age group and as a threshold of the Hb value: children <5 years old (group A): Hb < 110 g/l; 5-12 years (group B): Hb < 115 g/l; boys of 12-15 years (group C) as well as girls of 12-15 years (group D): Hb < 120 g/l; non-pregnant females above 15 years (group E): Hb< 120 g/l and males above 15 years (group F): Hb <130 g/l. Severe anemia was define as an Hb value of <70g/l (WHO/UNICEF/UNU, 2001).

Serum ferritin was only measured in 2000. A sample of 3 ml of venous blood was collected from all subjects and centrifuged immediately. Sera were stored at -70ºC until measurement in the Academic Medical Center, the Netherlands. Iron deficiency in both sexes was defined as a ferritin level of <12 μg/l for children <5 years old, and <15 μg/l for the other subjects. Iron deficiency anemia (IDA) was anemia plus iron deficiency (WHO/UNICEF/UNU, 2001).

Analysis

All data were entered into a computerized database with the repeated measurements of Hb, malaria, and intestinal helminth infections specified for all inhabitants. Data were analyzed on the basis of repeated measures.

The associations between anemia and malaria, or intestinal helminth infections, were investigated by year with Student's *t*-test for Hb values, using SPSS (version 11.0, SPSS Inc, Chicago, Ill). For the longitudinal analysis, generalized estimating equations was applied with "anemia" as the repeated

Table 1
The mean Hb values and prevalence of anemia in Phan Tien from 1996 to 2000, stratified by age and gender.

Year	Hb levels by age group						Proportion of anemia/ severe anemia (%)
	<5 Mean (SE)	5-12 Mean (SE)	12-15 boy Mean (SE)	12-15 girl Mean (SE)	>15 male Mean (SE)	>15 female Mean (SE)	
1996	99.7 (1.3)	105.4 (1.4)	121.3 (3.8)	112.7 (2.6)	110.9 (1.2)	128.3 (1.6)	66.1 (2.1)
Apr 1994	103.8 (1.7)	111.8 (1.2)	110.6 (3.0)	118.6 (2.5)	112.0 (3.5)	112.2 (6.5)	62.6 (1.2)
Nov 1997	98.1 (2.2)	105.3 (1.1)	110.7 (2.2)	108.6 (2.1)	107.8 (1.4)	122.1 (1.9)	76.8 (3.0)
1998	104.2 (1.7)	116.9 (1.1)	118.8 (3.5)	126.5 (1.6)	118.3 (1.6)	129.9 (3.2)	43.0 (2.2)
1999	104.6 (2.5)	115.6 (1.1)	119.8 (4.5)	121.0 (3.6)	119.5 (1.6)	129.3 (1.7)	47.8 (1.3)
2000	100.0 (1.3)	108.8 (1.1)	116.9 (3.2)	119.8 (2.8)	117.1 (5.0)	104.5 (6.5)	64.9 (2.4)

measure, using SAS (release 8.01, Sas Institute Inc, Cary, NC, USA). Since the value of Hb itself changes by age it was not analyzed in a repeated measures model. Statistical significance was accepted when $p<0.05$.

RESULTS

The results of the malaria control program in Phan Tien have been presented previously (Hung *et al*, 2002). In summary, during the first survey in 1994, 41% of the general population carried malaria parasites, of which *Plasmodium falciparum* contributed 70%. Following interventions with early diagnosis and treatment of malaria, distribution of insecticide treated bed nets, and health education, the malaria prevalence decreased dramatically. The overall prevalence of malaria over the study period is shown in Fig 1. In 1998, no malaria was detected in the indigenous population of Phan Tien. Although malaria was reintroduced by new settlers, road and forest workers, the overall prevalence still remained low (3%) in 1999. There was no indication of local malaria transmission in the commune itself.

The intestinal helminth infection control program has also been described elsewhere (Hung *et al,* 2005). In summary, in April 1997, intestinal helminth infections were diagnosed in 28.6% of the children of Phan Tien. Hookworm infections were the most frequent (23%), followed by *Trichuris trichiura* (1.9%), *Hymenolepis nana* (1.9%), *Enterobius vermicularis* (0.9%), *Ascaris lumbricoides* (0.5%), and multiple helminths infection (0.5%). Most of the mixed infections also involved hookworm. *Strongyloides stercoralis* was not detected. A control program, based on albendazole mass treatment, safe water supply, and health education, started after this survey. *Hymenolepis nana* infections were treated with praziquantel in 1998 and 1999. To ensure safe water supply, wells were made available at the end of 1997. Improvements of the sanitary facilities were carried out simultaneously, supported by health education and the promotion of personal and environmental hygiene.

The prevalence of intestinal helminth infections decreased significantly during the program, despite a delay during the first 6 months caused by using inappropriately formulated albendazole tablets. By the end of 1999, the overall prevalence of intestinal helminth infections had decreased to 3.3% and hookworm infections to 0.8%. The prevalence of intestinal helminth infections is shown in Fig 1.

Hb was measured in a total of 2,767 blood samples, collected during the 6 surveys. The prevalence of anemia and sever anemia in the first survey, in 1996, was 66.1% and 2.1%, respectively. The prevalence of anemia observed in the consecutive surveys is shown in Fig 1, along with malaria and intestinal helminth infections. Fig 2 presents all the Hb values from the first survey (1996), stratified by age and gender. The Hb values and anemia rates, specified by age group and survey, are shown in Table 1. During the consecutive six surveys, the mean Hb values of children in group A were often the lowest.

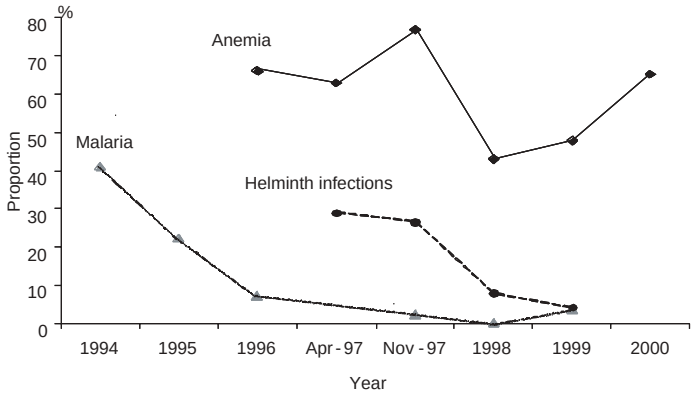

Fig 1–The prevalence of malaria, intestinal helminth infections, and anemia in the population of Phan Tien.

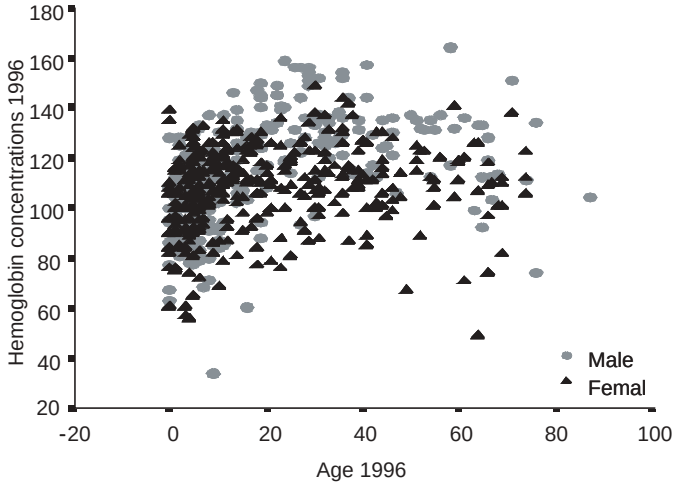

Fig 2–Distribution of hemoglobin concentrations in the population of Phan Tien in 1996, stratified by sex and age.

The prevalence of anemia in Phan Tien was very high, but variation was considerable, ranging from 43% to 77%, and severe anemia ranged from 1.2 to 3.0%. This is considered to be of severe significance from the perspective of public health (WHO/UNICEF/UNU, 2001).

The mean Hb values of subjects with and without malaria were compared with Student's *t*-test for every age group in every survey. Hb values were not different between parasitemic and non-parasitemic subjects in the surveys of 1996 through 1997 (data not shown). The Hb values of parasitemic subjects were lower in group A (t=3.49; p=0.001) and group B (t=2.99; p=0.02) in 1999, and in group B (t=2.61; p=0.01) in 2000. There were no significant associations between malaria and anemia rates among the different age groups. By analysing the repeated data with generalized estimating equations (GEE), a significant decline of the anemia prevalence was detected (OR: 0.805; 95% CI: 0.761 to 0.850, p<0.0001) with an independent significant effect of the malaria prevalence (OR: 2.408; 95% CI: 1.458 to 3.796, p=0.0006). In the subgroup of children younger than 17 years with available stool examination results, the same findings were confirmed, but there was no significant effect of the intestinal helminth infections on the time course of anemia (95% CI: -0.1548 to 0.1651).

Ferritin concentration was only measurred in 2000. The mean (± SE) ferritin values were 8.9 μg/l (± 9.1) and 8.2 μg/l (± 5.2) for children ≤ 5 years old and the remaining population, respectively. In girls and females, ferritin values were lower than in boys and males, but this did not reach statistical significance (data not shown). The prevalence of iron deficiency was very high: 86.3% of the overall population. Iron deficiency was found in 85.7% of anemic subjects.

DISCUSSION

This study uncovered a high frequency of anemia in an ethnic minority commune in Vietnam. Anemia in developing countries is often related to malaria and hookworm infections. In this commune, anemia was not easily redressed by controlling both infections. Understanding the dynamics of anemia, malaria, and hookworm infections as a public health problem may benefit public health intervention programs.

The prevalence of anemia varied during the study period, but nearly half of population always suffered from anemia. Initially, malaria was considered the most significant cause. It is evident that malaria causes hemolysis, and that both symptomatic and asymptomatic malaria are strong risk-factors for anemia (Hedberg *et al*, 1993; Cornet *et al*, 1998). In this study, we did

not detect an association between malaria and hemoglobin concentrations, or even anemia, when analyzing the respective surveys by themselves, except in 1999. Not until the repeated dataset was analyzed with GEE, did the relationship between anemia and malaria become clear.

Similarly, Stoltzfus *et al* (1997, 2000) did not detect any association between malaria and hemoglobin concentration on Pemba Island, off the East African coast, except in children below 30 months of age with a high parasite density, probably because the statistical power of analyzing a single cross-sectional survey is limited.

In our study, eventually by 1999, when local malaria transmission had been interrupted for approximately two years, Hb levels were lower in malaria-infected subjects. This may suggest a possible role of malaria immunity also in protecting against anemia. An arithmetic, but more plausible, explanation is that in hyperendemic areas, such as Phan Tien was in the early 1990s, malariometric surveys only measured the point prevalence of parasite carriers. They underestimated the total force of infection and thus obscured the association with the chronic effects of repeated infections, such as anemia. However, the number of malaria cases during the survey of 1999 was too small to draw sound conclusions.

When the improvement of anemia did not substantiate, we postulated that hookworm infections were a significant contributor to anemia. This was confirmed when a high frequency of hookworm infections was demonstrated, but no association between hookworm infection and anemia was found. Also, control of hookworm infections did not improve the rate of anemia, despite the fact that most of the anemia was explained by iron deficiency. Worldwide, hookworm infections are an important cause of iron deficiency, especially the infections with high-worm densities (Robertson *et al*, 1992; Hopkins *et al*, 1997; Olsen *et al*, 1998; Stoltzfus *et al*, 2000). In our study, we did not count egg loads, and so we cannot exclude the possibility that worm densities were low and thus did not contribute significantly to iron deficiency anemia.

The most likely explanation for the high rate of anemia in this population is therefore insufficient dietary intake of micronutrients, especially iron. It may explain a delayed recovery of iron deficiency anemia after the control of hookworm infections. In another study (Hung *et al,* 2005), we showed that the Phan Tien inhabitants had a marginal nutritional status throughout the study period. Previously we observed some reversal of stunted growth in Phan Tien, probably related to the control of malaria. This suggests that macronutrient deficiency was not critical but that micronutrients, especially iron, were deficient. Iron supplementation through existing control programs such as the malaria control program should be considered.

Hemoglobinopathies and thalassemia may also contribute to anemia. Thalassemia and hemoglobin E occur in Vietnamese subjects, but the prevalence in Vietnam is not known (Nguyen, 1990). Attempts to investigate this in Phan Tien have not been successful thus far.

In conclusion, in ethnic minority communes in Vietnam, anemia is highly prevalent, but it can not serve as a proxy indicator of malaria, nor of hookworm infections. This is probably caused by the marginal nutritional status of those who are at-risk of malaria and helminth infections. Since micronutrient deficiency is common in the vulnerable populations of Southeast Asia, infectious disease control programs should be accompanied by a monitoring of iron deficiency and, when needed, supplementation can be organized through the helminthiasis or malaria control programs.

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