Seroepidemiological Studies of Malaria in a Hypo and in a Mesoendemic Area, Indonesia

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Abstrak

Suatu studi cross sectional untuk mengetahui keadaan zat anti malaria kelas IgG penduduk daerah hipoendemi di Wonosobo, Jawa Tengah dan daerah mesoendemi di Flores, NTT, telah dilakukan dengan metoda ELISA. Antigen yang dipakai adalah ekstrak stadium skizon Plasmodium falciparum strain Flores yang dibiak secara in vitro. Hasil yang diperoleh menunjukkan adanya perbedaan yang bermakna antara zat anti malaria penduduk hipoendemi dibandingkan dengan zat anti malaria penduduk mesoendemi dalam hal angka sero-positif (18/282 versus 48/155), titer rata-rata geometrik zat anti (21.26 versus 31.27), dan rata-rata geometrik titer positif (48,48 versus 82,28). Dengan mengkaitkan gejala klinik dan tanda infeksi malaria yaitu splenomegali dan parasitemia dengan zat anti malaria tersebut, ternyata di daerah hipoendemi parasitemia lebih berperan, sedangkan di daerah mesoendemi splenomegalilah yang menonjol. Penelitian seroepidemiologi ini penting dilakukan karena lebih baik untuk menelaah keadaan penyakit malaria yang sebenarnya di suatu daerah dibandingkan dengan hanya menggunakan angka limpa dan/atau angka parasit saja.

Abstract

A cross-sectional study has been done in order to examine antimalarial antibody (IgG) from people living in hypoendemic and mesoendemic areas in Wonosobo, Central Java and Flores, East Nusa Tenggara with ELISA. The antigen used was the extract of Plasmodium falciparum schizonts (Flores strain) cultured in vitro. By using Student's t test, results obtained showed a significant difference concerning the seropositive rate between hypoendemic and mesoendemic area (18/282 vs 48/155), the antibody geometric mean titre (21.26 vs 31.27) and the geometric mean of the positive titre results (48.48 vs 82.28) ($p < 0.05$). By connecting clinical symptoms and signs i.e. splenomegaly and parasitemia with antimalarial antibody detection it was found that parasitemia was more prominent in the hypoendemic area, whereas splenomegaly was more prominent in the mesoendemic area. It was concluded that sero-epidemiology studies were more important than using spleen rate and/or parasite rate only to evaluate the malaria situation in an area.

Keywords : *IgG antibodies, ELISA, Cross-sectional, Schizont.*

INTRODUCTION

Assessment of the endemicity or the severity of disease in a malarious area can be done by determining the spleen rate and the parasite rate. Assessing the parasite rate i.e. the percentage of the parasite harbouring individuals showing "point prevalence" is easily done by examining thick blood film. However, undiagnosed cases are common because malaria parasites circulate in the peripheral blood for 4 weeks approximately and many cases have low parasitemia in endemic area due to immune reaction.[1]

Spleen rate can assess the endemicity of an area; this data showed "period prevalence" because the spleen will remain enlarged for several weeks although parasites are no longer found in peripheral blood. This method is preferred over assessing the parasite rate, although it has some limitations : it is difficult to palpate a soft spleen in acute malaria. Moreover, other parasitic diseases as well as non parasite diseases can also cause spleen enlargement.[2.]

In 1974 WHO suggested to use a serological test to detect specific antimalarial antibody in order to assess the endemicity of an area.[3.] This method can show the "period prevalence" because the antibody will remain circulating in the blood for several months. Some investigators working in the various endemic areas in the world said that this serological test can give a more reliable picture concerning the malaria disease in an endemic area.[4,5,6.]

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Seroepidemiological studies are seldom done in Indonesia, whereas epidemiological studies in the field use only parasite rate and spleen rate parameters. The objective of this study is to know whether a cross-sectional seroepidemiological study can differentiate two areas with different endemicity and the relation between antibody detection and parasite rate, spleen rate and fever in these areas.

MATERIALS AND METHODS

A survey has been done in two malaria endemic areas in 1990, in Wonosobo, Central Java and in Flores, East Nusa Tenggara. Spleen examination was done according to the Hacket's method and blood examination was performed by thick smear stained with Giemsa. Fever was confirmed if during physical examination the body temperature was higher than 37.5°C or the patients have had fever about a week before according to anamnesis. Based on spleen rate in children between 2-9 years old, Wonosobo was a hypoendemic area (6.3%) and Flores was a mesoendemic area (27.1 %). *P. falciparum* was the dominant species in Flores, with the coexistence of *P. vivax*; whereas in Wonosobo, Central Java *P. falciparum* was the only species of Plasmodium.

Venous blood collection have been done for serological studies. After centrifugal separation the sera were stored in -20°C until used. In Wonosobo, Central Java, 282 sera (80.3%) and in Flores, 155 sera (79.1%) have been collected from the examined inhabitants.

Antigen was made from *P. falciparum* Flores's strain, which have been cultured *in vitro* according to the method of Trager and Jensen.[7] When the density of parasitemia has reached 10%, the schizont stages were isolated and disrupted with an "ultrasonic desintegrator" for 5 minutes on ice, then centrifuged at 9000 g for 30 minutes at 4°C. The supernatant was used as antigen after protein determination. The method of ELISA used was according to the previous study.[8] The result was positive when at 1 : 40 sera dilution the O.D. value at 490 nm was more than 0.29.

Statistical analysis was done by using Student's t test and chi-square with Yate's correction whenever necessary with $p < 0.05$ as a limit of significant differences.

RESULTS

Parasite rate, splenomegaly, fever rate and seropositive rate in a hypoendemic area

From 282 persons in Wonosobo, Central Java, seropositive rate (6.3 %) was almost equal to fever rate (6.7 %), but higher compared to the parasite rate (5.3%) and significantly higher compared to the splenomegaly (2.4%) (Table-1). A graph based on this 4 parameters, showed a fluctuation pattern (Table-1, figure-1). Parasite rate (3/14 = 21.4 %) and the splenomegaly (2/14 =14.2 %) were highest in age group under 5 years, fever was found in all age groups, but the highest rate was also in age group under 5 years (21.4 % = 3/14) and in the 16-20 years (20 % = 2/10) (Table-1, figure-1).

Parasite rate, splenomegaly, fever rate and seropositive rate in a mesoendemic area

Out of 155 persons surveyed in Flores, the seropositive rate (30.9 %) was almost equal to the splenomegaly (31.6 %), whereas the parasite rate and fever rate were only 8.3 % and 5.1 % respectively (Table-2). Splenomegaly and seropositive rate in the younger group (under 15 years and 10 years) tend to fluctuate and then slowly increased until a peak at 66.5 % for splenomegaly and 81.8 % for seropositive rate in the 31 - 40 years old age group (Figure-2). These 2 parameters then decreased on the following age groups. By contrast, the peak of parasite rate and fever rate were under 10 years old, whereas only 2 persons suffered from fever and 2 persons had parasitemia in the over 10 years age group (Table-2, figure-2).

Comparison of serological responses between hypo- and mesoendemic inhabitants

There was a significant difference between seropositive rate of hypoendemic and mesoendemic inhibitants (6.3 % and 30.9 %) ($p < 0.05$) (Student's t test).

The geometric mean titer of hypoendemic inhabitants (21.26) also was significantly different compared to the values of mesoendemic inhabitants (31.27) ($p < 0.05$) (Student's t test).

The geometric mean positive titer of hypoendemic inhibitants (48.48) showed a significant difference between mesoendemic inhabitants (82.28) ($p < 0.05$) (Student's t test). No age classification significant test was done for this value because some age groups of hypoendemic inhibitants showed negative results.

Table 1. Classification by age of parasite rates, splenomegaly, fever rate, seropositive rates and antibody geometric mean titre of villagers in a hypoendemic area, Wonosobo, Central Java.

Age (years)	N	Parasite rate (%)*	Splenomegaly (%)*	Fever rate (%)*	Seropositive (%)*	Geometric Mean Titre of Antibody (GMT)
0 - 5	14	21.4 %	14.2 % (2/14)	21.4 % (3/14)	7.1 % (1/14)	23.19
6 - 10	56	3.5 %	3.5 % (2/56)	12.5 % (7/56)	7.2 % (4/56)	21.53
11 - 15	45	4.4 %	2.2 % (1/45)	2.2 % (1/45)	0 % (0/10)	19.19
16 - 20	10	10 %	0 % (0/10)	20 % (2/10)	0 % (0/10)	19.19
21 - 25	16	12.5 %	6.2 % (1/16)	6.2 % (1/16)	6.2 % (1/16)	21.80
26 - 30	19	0 %	0 % (0/19)	5.2 % (1/19)	0 % (0/19)	19.99
31 - 35	14	0 %	0 % (0/14)	14.2 % (2/14)	14.3 % (2/14)	22.08
36 - 40	17	0 %	0 % (0/17)	5.9 % (1/17)	5.9 % (1/17)	20.38
> 40	91	5.5 %	1 % (1/91)	1 % (1/91)	9 % (9/91)	22.19
Total	202	5.3 %	2.4 % (7/282)	6.7 % (19/282)	6.3 % (18/282)	21.16

$$* \% = \frac{\text{Total positive}}{\text{Total age group}}$$

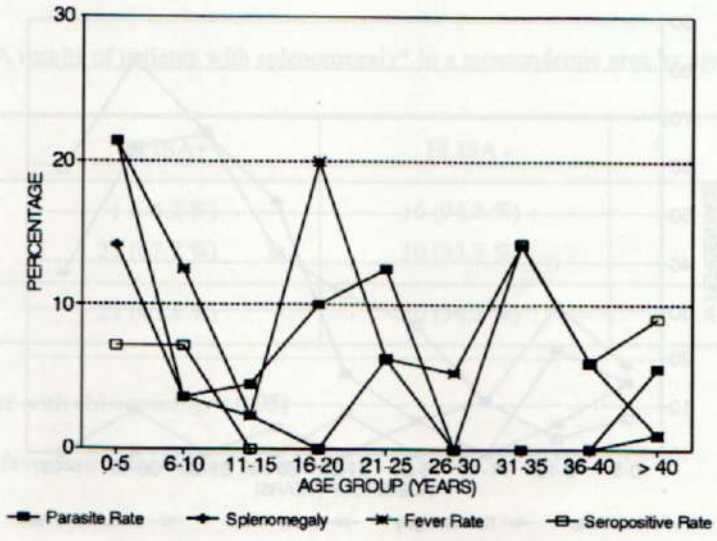

Figure 1. Parasite rate, splenomegaly, fever rate, seropositive rate in a hypoendemic area

Table 2. Classification by age of parasite rate, splenomegaly fever rate, seropositive rates and antibody geometric mean titre of villagers in a mesoendemic area of Flores, East Nusatenggara.

Age (years)	N	Parasite rate (%)*	Splenomegaly (%)*	Fever rate (%)*	Seropositive (%)*	Geometric Mean Titre of Antibody (GMT)
0 - 5	27	14.8 %	18.5 % (5/27)	14.8 % (4/27)	7.4 % (2/27)	24.55
6 - 10	32	21.8 %	31.2 % (10/32)	6.2 % (2/32)	3.2 % (1/32)	21.34
11 - 15	9	0 %	11.1 % (1/9)	0 % (0/9)	11.1 % (1/9)	21.60
16 - 20	15	6.6 %	6.6 % (1/15)	0 % (0/15)	26.7 % (4/15)	27.63
21 - 25	12	0 %	16.6 % (2/12)	8.3 % (1/12)	33.3 % (4/12)	37.75
26 - 30	19	5.2 %	52.6 % (10/19)	0 % (0/19)	42.1 % (8/19)	38.56
31 - 35	6	0 %	66.6 % (4/6)	0 % (0/6)	66.8 % (4/6)	63.49
36 - 40	11	0 %	63.6 % (7/11)	9 % (1/11)	81.8 % (9/11)	48.31
> 40	24	0 %	37.5 % (9/24)	0 % (0/24)	59 % (15/24)	42.94
Total	155	8.3 %	31.6 % (49/155)	5.1 % (8/155)	30.9 % (48/155)	31.27

$$* \% = \frac{\text{Total positive}}{\text{Total age group}}$$

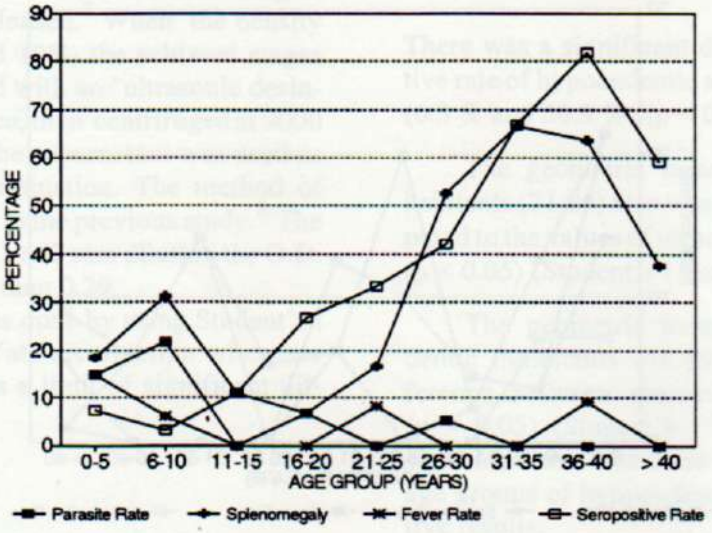

Figure 2. Parasite rate, splenomegaly, fever rate, seropositive rate in a mesoendemic area

Comparison of ELISA results between hypo- and mesoendemic inhibitants classified by age

There was no significant difference on seropositive rate between hypoendemic inhabitants under 20 years compared to inhabitants over 20 years (Table-3, $p < 0.05$, chi-square).

By contrast, there was a significant difference of the seropositive rate between mesoendemic inhabitants under 20 years compared to inhabitants over 20 years (Table-3, $p < 0.05$, chi-square). Comparison of the seropositive rate of inhabitants over 20 years of a hypo and a mesoendemic area showed a significant difference (Table-3, $p < 0.05$, chi-square).

Comparison of ELISA on patients with splenomegaly in a mesoendemic area classified by age

There was a significant difference of the seropositive rate of people with splenomegaly under 20 years compared to people with splenomegaly over 20 years (Table-4, $p < 0.05$, chi-square).

Table 3. Comparison of ELISA results of villagers in a hypo- and a mesoendemic area by age group

Classification	Area	ELISA +	ELISA -	Total
Age < 20 yrs	hypo-endemic	5 (4 %) A	120 (96 %)	125
	mesoendemic	8 (9.6 %) B	75 (90.4 %)	83
Age ≥ 20 yrs	hypo-endemic	13 (8.2 %) C	144 (91.8 %)	157
	mesoendemic	40 (55.5 %) D	32 (45.5 %)	72

A & B → no significant difference with chi-square ($p > 0.05$)
C & D → significant difference with chi-square ($p < 0.05$)
A & C → no significant difference with chi-square ($p > 0.05$)
B & D → significant difference with chi-square ($p < 0.05$)

Table 4. Comparison of ELISA results of patients with splenomegaly* in a mesoendemic area by age group

Classification	ELISA+	ELISA -	Total
Age < 20 yrs	1 (6.2 %)	16 (94.8 %)	17 (100 %)
Age ≥ 20 yrs	21 (67.7 %)	10 (33.3 %)	31 (100 %)
Total	22 (45.8 %)	26 (54.2 %)	48 (100 %)

There is a significant difference with chi-square ($p < 0.05$)

* Splenomegaly with/without fever and with/without parasitemia.

DISCUSSION

Development of natural acquired immunity in malaria endemic areas is a long process which perhaps requires years and is based on continuation of host exposure to the parasite itself. If the host leaves this area for a period of time, then he or she might lose his or her immunity and on returning to the endemic area would probably be vulnerable again. This process is called premunition.[9]

Our study in a hypoendemic area showed a fluctuation of the malaria antibody level (Figure-1). This graph occuring in the population. This observation was also supported by the fluctuation of splenomegaly, parasite rate and fever rate (Figure-1, Table-1). The relevancy of serological responses and malaria infection has been reported also by other authors.[5,6,10] The high level of parasite rate and splenomegaly in the age group below 5 years was difficult to interpret, considering the difficulty of collecting venous blood in this age group. The possibility of disparity of immune responses among different age groups was difficult to demonstrate, due to the insignificant differences of seropositive rates and the geometric mean titres of antibodies, which showed nonaccumulative antibodies of natural immunity; though the relevancy of these detected protective antibodies still have to be verified.

Interpretation of fever as one of the main symptoms of malaria has to be done deliberately, since the cause of fever could be other agents, besides some of the data was collected by anamnesis. Results from the hypoendemic area revealed only 5 out of 20 patients who have had fever had parasitemia (data not shown), although the possibility of low density parasitemia could not be exclusively excluded. Moreover, the percentage of fever rates both in meso and hypoendemic areas (5.1 % and 6.7 %) were almost equal suggesting that fever in an area with a higher endemicity level is not longer an important malaria symptom. In hypoendemic areas without natural acquired immunity, epidemics could happen anytime especially at high transmission seasons. This will give a bad impact both on children and older age groups.

By contrast, in the mesoendemic area, the seropositive rate and the geometric mean titre increased according to age (Figure-2) and showed a significant difference of these two parameters between group over 20 years and group under 20 years. This was also confirmed by splenomegaly. On the other hand, parasitemia and fever cases were mostly found in the age group under 20 years, in the period where natural acquired immunity has not been developed. All patients with splenomegaly and parasitemia were of the under 20 years group and only one person (5.8 %) had positive antibody based on ELISA (data not shown). On the other hand, not any patient with splenomegaly over 20 years had parasitemia, but 67% of them had malaria antibodies (Table-4). This data revealed the possibility of obtaining natural acquired immunity in patients with splenomegaly of over 20 years. The role of the spleen for protection in malaria infection has been described by other authors.[11] Whether malaria antibodies detected in this study play an important role in host defense mechanism still has to be evaluated by further studies.

It was concluded that further seroepidemiological studies on hyper- and holoendemic areas are necessary to provide significant information for the planning of malaria control programmes in Indonesia.

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