Estimation of malaria parasite densities by different formulas in Thailand

Abstract

Introduction: Although there are many methods in malaria diagnoses e.g., quatitative buffy coat (QBC), rapid diagnosis tests (RDTs), serological tests and molecular diagnosis methods such as PCR, but microscopy still remains the gold standard for malaria diagnosis. Estimation of malaria parasite density can be carried out by using assumed white blood cells (WBC) and red blood cells (RBC) counts.

Objective: The aims of this study were to determine malaria parasite densities calculated by assumed WBC and RBC counts; and to compare their reliability with absolute WBC and RBC counts.

Methods: The clinical presentations and laboratory findings of specimens collected from 512 uncomplicated falciparum and vivax malaria patients admitted to Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand were utilized and analysed for estimation of malaria parasite densities by using different formulas.

Results: Parasite densities calculated by WHO recommended assumed WBC of 8,000 /µL, and assumed RBC counts of $4.7x10^6$-$6.1x10^6$ /µL and $4.2x10^6$-$5.4x10^6$ /µL for males and females respectively led to overestimation, and resulted in low reliability when compared to the absolute WBC and RBC counts. Parasite densities calculated by assumed WBC of 5,900/µL in thick blood; by assumed RBC of $4.8x10^6$/µL for male and $4.3x10^6$/µL for female in thin blood film respectively gave more precise estimation.

Conclusion: Assumed WBC and RBC counts for calculating malaria parasite densities have to be adjusted to use in Thailand for more precise estimation. Parasite densities calculated by assumed WBC and RBC used in other malaria endemic countries might need further re-evaluation.

Keyword:

Malaria, parasite, density, estimating, formulas, Thailand

Introduction

Malaria is the most important blood-borne protozoan disease of human transmitted by female *Anopheles* mosquitoes. In 2017, an estimated 219 million patients of malaria occurred worldwide and most 200 million malaria patients were in the WHO African Region, followed by the WHO South-East Asia Region with 5% of the patients and the WHO Eastern Mediterranean Region with 2% [1]. There were an estimated 435,000 deaths from malaria globally in 2017 [1]. Early diagnosis and treatment is crucial in management of malaria. Parasitological diagnosis carried out by conventional microscopy remains gold standard for malaria diagnosis, although, there are many modern methods to diagnose malaria nowadays. Microscopy can also estimate parasite density in patients' own white blood cells (WBC) and red blood cells (RBC) by thick and thin blood films respectively. Determining parasite density level is important in severity classification, clinical management, monitoring drug efficacy and predicting prognosis of malaria. Currently, there are different counting methods and calculation formulas of parasite density. Although using absolute counts of WBC and RBC of a patient is more accurate in parasite density estimation, assumed counts of WBC and RBC recommended by WHO [2,3] are widely used in many malaria endemic areas because automated hematological analyzers (AHAs) are expensive and required regular maintenance, reliable power supply, and trained operators Thus, they are unavailable in many health facilities in those areas. Assumed WBC counts of 8,000/µL was the average WBC value of an African country, Nigeria [4]. Technical reports from Africa, South America and Papua suggested that parasite densities calculated by this assumed WBC count might be underestimated, similar, or overestimated comparing with those calculated by assumed WBC count [5].

The aims of this study were to clarify assumed WBC and RBC counts in order to estimate malaria parasite densities; and compare them with those calculated from absolute WBC and RBC counts (derived from AHAs).

Materials and Methods

Study Site and Enrollment Procedures

This study was conducted at the Hospital for Tropical Diseases (HTD), a tertiary healthcare facility, in Bangkok, Thailand. Patients meeting the following criteria were included in the study: (i) males or females, aged $\geq$15 years; (ii) admitted for the treatment of falciparum or vivax malaria (iii) microscopically confirmed diagnosis for asexual-stages of either uncomplicated *P. falciparum* or *P. vivax* mono-infection (iv) conducted complete blood count (including absolute WBC and RBC) by AHAs upon admission; (iv) no history of antimalarial therapy during a month prior to admission. We excluded severe malaria patients regarding to WHO (2015) [5] and patients with histories of significant concomitant diseases.

Clinical Management, Laboratory Investigations, and Data Collection

Patients were evaluated and managed according to the standard hospital practice. Falciparum malaria patients were treated with oral artemisinin-based combination therapies (ACTs). Vivax malaria patients were treated by oral chloroqune followed by primaquine for hypnozoite eradiacation. Parasite density levels (ring to schizont forms) were evaluated using thick and thin blood films stained with Giemsa. Baseline clinical manifestations, demographic information, and laboratory data were examined and recorded. The parasite density of asexual forms/µL was calculated from (i) absolute WBC and RBC derived from AHAs, (ii) using WHO recommended assumed WBC count of 8,000 /µL [2] and other assumed WBC /µL, (iii) using assumed RBC count of $5x10^6$ /µL (for male), $4.5x10^6$ /µL for female), and other assumed RBC counts. Therefore, parasite density was calculated as follows:

$$\text{Parasite density/µL} = \frac{\text{No. of parasites counted}}{\text{No. of WBC counted}} \times \text{absolute or assumed WBC of patient}$$

or

$$\text{Parasite density/µL} = \frac{\text{No. of parasite counted}}{\text{No. of RBC counted}} \times \text{absolute or assumed RBC of patient}$$

Ethical Approval

This study was approved by the Ethics Committee of Tropical Medicine, Mahidol University, Bangkok, Thailand (Approval number: MUTM 2014-064-01.)

Statistical Analysis

Statistical analysis was carried out using SPSS for Windows, version 16. Quantitative data was tested with Wilcoxon signed-rank test to compare two related samples, Man-Whitney *U* test for difference between two groups and Kruskal-Wallis test for more than two groups of patients. Reliability analysis was carried out to measure the overall consistency of the items that were used to define a scale. The Wilcoxon signed-rank test was used to compare paired patient data with a two-tailed significance level of P <0.05.

Results

Demographic data of studied patients

Among 512 cases of malaria infected patients, 425 (83%) and 87 (17%) patients were males and females respectively (Table 1). These patients were aged between 14 and 76 years with a median age of 25 years at presentation. Patients in 2nd decade (≤ 20 years) and 3rd decade of life (21-30 years) were 181 (35.4%) and 205 (40%) respectively accounting for 75.4% of studied population. The rest of the patients (24.6%) had age >40 years. Regarding to ethnicity, 42 (8.2%) patients were Thai whereas 312 (60.9%), 101 (19.7%), 51 (10%), 3 (0.6%) and 3 (0.6%) were Myanmar, Karen, Mon, Laos, and Cambodian respectively. Among the 512 patients, 251 (49%) were infected with *P. falciparum* and 261 (51%) with *P. vivax*. There were 204 (48%) and 47 (54%) male and female patients respectively. Out of 425 male patients, there were 204 (48%) infected with *P. falciparum* and 221 (52%) with *P. vivax*. In 87 female patients, there were 47 (54%) and 40 (46%) infected with *P. falciparum* and *P. vivax* respectively.

WBC counts

Mean of absolute WBC was (6,051± 1,954)/µL in the studied population. 71 (13.9%) patients had leukopenia whereas 432 (84.4%) patients had normal WBC. Leukocytosis was observed only in 9 (1.8%) patients. The normal range for WBC counts in most laboratories were 4,000-11,000/µL.

Absolute RBC counts

Mean of absolute RBC counts was (4,632,227± 815,103)/µL. Normal range of RBC counts in most laboratories were 4.5x10^6-5.8x10^6/µL in male and 4.2x10^6-5.4x10^6 RBC/µL in female respectively [3,6]. RBC counts of 306 (60%) patients were normal whereas 178 (35%) patients had reduced. Increased RBC counts were found in 28 (5%) patients.

Table 2 showed absolute RBC counts were different between male and female (P <0.001), and *Plasmodium* species (P <0.001). The RBC counts of the male patients were higher than of female patients. RBC counts of falciparum malaria patients was lower than of vivax patients. RBC counts were different (P <0.001) among ethnic groups. Thai patients had higher RBC counts than Myanmar, Karen, and Mon (P <0.001). RBC counts in Myanmar patients were also higher than Karen (P=0.044) and Mon (P=0.036) ethnic groups.

Parasite counts

Among 512 samples, asexual forms of *P. falciparum* and *P. vivax* were found in 251 and 261 patients respectively. Table 3 showed both parasite densities calculated from using absolute WBC derived from AHAs and assumed WBC count of 8,000 µ/L [2] with 200 WBC microscopy counted respectively.

Parasite density estimated by assumed WBC count of 8,000/µL compared with absolute WBC counts showed that 439 (85.7%) patients were overestimated; and 70 (13.7%) patients were underestimated with ($P < 0.001$). Estimating parasite densities by other assumed WBC counts of 4,000; 5,000; 6,000; 7,000; 9,000 and 10,000/µL were shown in Table 4.

Assumed WBC counts of 4,000 and 5,000/µL showed significantly underestimated ($P < 0.001$) and assumed WBC counts of 7,000; 8,000; 9,000 and 10,000/µL showed significantly overestimated ($P < 0.001$). Parasite density calculated by assumed WBC count of 6,000/ µL did not show significantly different from parasite density calculated by absolute WBC count (Table 5).

To obtain a more precise assumed WBC value to estimate parasite density, parasite density was estimated by assumed WBC counts of 5,500; 5,800 and 5,900/µL and compared with parasite densities calculated by absolute WBC counts (Table 6).

Table 7 showed estimated parasite densities calculated with assumed WBC counts of 5,800; 5,900 and 6,000 WBC/µL were similar to absolute parasite densities. Parasite density estimated with assumed WBC count of 5,900 showed the most similar value ($P=0.925$) with absolute parasite density.

Assumed RBC counts

Parasite densities calculated by using absolute RBC count and estimating parasite densities calculated by assumed RBC counts from $4.7x10^6$-$5.2x10^6$ RBC/µL were shown in Table 8. Since reference values of RBC counts are not the same between males and females, parasite densities between male and female patients were estimated separately by assumed RBC count.

In Table 9, parasite density estimation with assumed RBC counts of $4.7x10^6$ /µL and $4.8x10^6$ /µL showed no significant difference with absolute RBC counts ($P=0.126$ and 0.608 respectively). Assumed RBC count of 4.8×10^6/µL showed mostly similar to parasite density calculated with absolute RBC count.

In females, parasite densities calculated by absolute RBC and assumed RBC counts from $4.2x10^6$-$4.7x10^6$ /µL was shown in Table 10. Parasite density estimations were highly reliable between assumed RBC counts of $4.2\ x10^6$-$4.4x10^6$/µL.

Table 11 showed assumed RBC counts $\geq$ $4.6x10^6$ RBC/µL were found to be significantly overestimated ($P < 0.01$) in parasite density. Assumed RBC counts of 4.2 $x10^6$/µL-$4.5x10^6$/µL showed no significantly different parasite density calculated by absolute WBC count ($P > 0.05$). Assumed RBC 4.3 $x10^6$/µL showed the most similar to absolute parasite density in females (with highest P value in Table 11).

Discussion

The median age in this study was 25 years (range 14-76 years) showing that malaria infection could occur in any age groups but it more commonly occurred in those aged 21-30 years old. The WBC count was similar between gender, and *Plasmodium* species whereas RBC count was significantly higher in male and vivax malaria patients.

When calculating parasite density by absolute WBC counts of patients in thick films, mean parasite density in this study was 20,826 parasites/µL (range 16-386,780 parasites/µL). In the study conducted in Brazil, mean parasite density was 7,519 parasites/µL (range 31-64,930 parasites/µL) calculated from absolute WBC counts [7]. The study in Ghana, mean parasite density was 49,474 parasites/µL (range 15-4,036,350) parasites/µL calculated by absolute WBC counts [8]. Parasite density estimated by assumed WBC 8,000/ µL [2] showed overestimation in comparing with parasite density estimated by absolute WBC count. Similarly, the study of children patients aged 1-8 years in Nigeria [9], study of African children <5 years [10] , and the study of mostly adults in Brazil [7], showed overestimation of parasite densities when they used WHO recommended WBC count of 8,000 cells/µL to estimate parasite densities. Assumed WBC counts of 5,500 cells/ µL [7] and 5,100 cells/µL [9] respectively could estimate parasite density more precisely. Studies in Ghana [8] and Sudan [11] mentioned underestimation of parasite density when assumed WBC count of 8,000/µL was used. Assumed WBC count of 10,000 cells/µL could estimate parasite density more precisely [8]. However, the study conducted in Papua New Guinea [12], parasite density estimation using assumed WBC of 8,000 cells/µL showed no significat difference with parasite density calculated by absolute WBC counts.

In this study, parasite densities estimated by other assumed WBC counts of 4,000 and 5,000/µL showed significantly underestimated ($P < 0.001$) and by assumed WBC counts of 7,000; 8,000; 9,000 and 10,000/µL showed significantly overestimated ($P < 0.001$) comparing with calculation by absolute WBC count. However, assumed WBC count of 6,000/µL showed no significantly different parasite density calculated by absolute WBC count. To obtain a more precise assumed WBC value to estimate more precise parasite densities, assumed WBC counts of 5,500; 5,800 and 5,900 WBC/µL were used for estimation and showed similar to absolute parasite density calculated by absolute WBC. Parasite density estimated with assumed WBC count of 5,900 showed the most similar value ($P=0.925$) with absolute parasite density, therefore it might be the most reliable assumed WBC count in this studied population. The possible reason that precisely assumed WBC count in Thailand was lower than WHO recommended assumed WBC might be due to general infections in people living in Thailand less than in African country [4] particularly in the past where WHO recommended to use assumed WHO count for malaria density estimation.

In this study, parasite density by assumed RBC count of 5.0 $x10^6$/µL (for males) and 4.5 $x10^6$ /µL (for females) showed overestimation, possibly people living in Thailand including Thai and other ethnicities from Myanmar had underlying anemia due to hemoglobinopathy (which is commonly found) [13, 14] and intestinal parasitic infection [15-19] causing lower exactly assumed RBC counts in these population in Thailand.

Conclusion

The application of assumed WBC count of 8 000 cells/µL and assumed RBC counts of 5.0 $x10^6$ /µL and 4.5$x10^6$ /µL for males and females respectively to estimate parasite densities in malaria patients led to overestimated parasite densities and resulted in low reliability when compared to absolute WBC and RBC counts from the AHAs. Calculating by new assumed WBC 5,900/µL in thick blood film; assumed RBC counts of 4.8 $x10^6$ /µL and 4.3 $x10^6$ /µL for male and female patients respectively in thin blood films for estimating parasite densities will provide more precised parasite densities in Thailand where malaria is endemic. However, assumed WBC and RBC counts may differ in other countries due to other national normal WBC and RBC values which are effected by many factors in different population in the world.

Competing interests

We declare that no competing interests exist.

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Table 1. WBC counts among gender, parasite species, and ethnicity in Thailand

Characteristics (N)	WBC/µl (SD)	P-value
Gender		
males (425)	6,000 (1,938)	0.308
females (87)	6,301 (2,023)	
Malaria species		
P. falciparum (251)	6,001 (2,009)	0.453
P. vivax (261)	6,100 (1,902)	
Ethnicity		
Thai (42)	5,705 (2,047)	0.111
Myanmar (312)	6,070 (1,936)	
Karen (101)	5,853 (1,878)	
Mon (51)	6,602 (2,123)	

Table 2. Absolute RBC counts among gender, parasite species, and ethnicity in Thailand

Characteristics (N)	RBC/µl (SD)	P-value
Gender		<0.001
Male (425)	4,705,271 (814,376)	
Female (87)	4,275,402 (723,041)	
Malaria species		<0.001
P. falciparum (251)	4,489,203 (848,185)	
P. vivax (261)	4,769,770 (758,485)	
Ethnicity		<0.001
Thai (42)	5,096,667 (897,190)	
Myanmar (312)	4,644,103 (802,786)	
Karen (101)	4,493,366 (721,759)	
Mon (51)	4,464,510 (864,850)	

N=number; RBC=red blood cells; SD= standard deviation

Table 3. Parasite densities calculated with absolute WBC counts in falciparum and vivax malaria patients

Parameter	Parasites/µL in falciparum malaria patients (N=251)	Parasites/µL in vivax malaria patients (N=261)
Minimum	16	28
25 Percentile	587	3,625
Median	9,040	10,800
75 Percentile	39,520	21,280
Maximum	386,780	115,000
Mean	26,917	14,968
Std. Deviation	42,231	16,336
Std. Error of Mean	2,666	1,011
Lower 95% CI of the mean	21,667	12,977
Upper 95% CI of the mean	32,167	16,960
Geometric mean of parasite density	4,256	4,254

Table 4. Parasite density calculated with absolute and assumed WBC counts from 4,000-10,000/µL (N=512)

Parameter	**Absolute WBC/µL**	**Assumed WBC/µL**						
		4,000	**5,000**	**6,000**	**7,000**	**8,000**	**9,000**	**10,000**
Minimum	16	20	25	30	35	40	45	50
25 Percentile	1,036	765	956	1,148	1,339	1,530	1,721	1,913
Median	10,300	7,370	9,213	11,055	128,898	14,740	16,583	18,425
75 Percentile	25,038	17,607	22,009	26,411	30,812	35,214	39,616	44,018
Maximum	386,780	166,357	207 946	249,535	291,125	332,714	374,303	415,892
Mean	20,826	14,398	17,998	21,598	25,197	28,797	32,396	35,996
Std. Deviation	32,312	21,903	27,379	32,855	38,331	43,806	49,282	54,758
Std. Error of Mean	1,428	968	1,210	1,452	1,694	1,936	2,178	2,420
Lower 95% CI of the mean	18,021	12,497	15,621	18,745	21,869	24,993	28,117	31,242
Upper 95% CI of the mean	23,632	16,300	20,375	24,450	28,525	32,600	36,675	40,750
Geometric mean	4,250	2,931	3,702	4,477	5,256	6,038	6,823	7,611

N=number; WBC=white blood cells; SD= standard deviation

Table 5. Underestimated and overestimated parasite density calculated with different assumed and absolute WBC as the standard (N=512)

Assumed WBC WBC/µL	**Underestimated *N***	**Overestimated *N***	**P-value**
5,000	348	156	<0.001
6,000	233	270	0.316
7,000	141	366	<0.001
8,000	70	439	<0.001
9,000	37	473	<0.001
10,000	17	495	<0.001

WBC=white blood cells

Table 6. Parasite density calculated with absolute and assumed WBC counts from 5,000 to 6,000 µL (N=512)

Parameter	**Absolute**	**Assumed WBC/µL**

	WBC/µL	5,000	5,500	5,800	5,900	6,000
Minimum	16	25	28	29	30	30
25 Percentile	1,036	956	1,052	1,109	1,128	1,148
Median	10,300	9,213	10,134	10,687	10,871	11,055
75 Percentile	25,038	22,009	24,210	25,530	25,970	26,411
Maximum	386,780	207,946	228,741	241,218	245,377	249,535
Mean	20,827	17,999	19,799	20,879	21,239	21,599
Std. Deviation	32,311	27,378	30,116	31,759	32,306	32,854
Std. Error of Mean	1,428	1,210	1,331	1,404	1,428	1,452
Lower 95% CI of the mean	18,022	15,622	17,184	18,121	18,434	18,746
Upper 95% CI of the mean	23,633	20,376	22,414	23,636	24,044	24,451
Geometric mean	4,250	3,702	4,089	4,322	4,399	4,477

WBC=white blood cells

Table 7. Underestimated and overestimated parasite density calculated with different assumed WBC counts from 5,000 -7,000 /µL with the absolute WBC counts as the standard (N=512)

Assumed values	Underestimated	Overestimated	P-value
5,000	348	156	<0.001
5,500	298	203	<0.001
5,800	259	246	0.343
5,900	245	253	0.925
6,000	233	270	0.316
7,000	141	366	<0.001

WBC=white blood cells

Table 8. Parasite density calculated with absolute and assumed RBC counts from $4.7x10^6$-$5.2x10^6/\mu L$ in male patients (N=280)

Parameter	**Absolute RBC/µL**	**BCx10^6/µl**					
		4.7	**4.8**	**4.9**	**5.0**	**5.1**	**5.2**
Minimum	2,160	4,700	4,800	4,900	5,000	5,100	5,200
25 Percentile	10,105	9,400	9,600	9,800	10,000	10,200	10,400
Median	17,430	18,800	19,200	19,600	20,000	20,400	20,800
75 Percentile	34,320	37,600	38,400	39,200	40,000	40,800	41,600
Maximum	386,780	390,100	398,400	406,700	415,000	423,300	431,600
Mean	29,687	29,274	29,897	30,520	31,143	31,766	32,389
Std. Deviation	36,211	34,947	35,691	36,434	37,178	37,922	38,665
Std. Error of Mean	2,164	2,089	2,133	2,177	2,222	2,266	2,311
Lower 95% CI of the mean	25,427	25,163	25,698	26,234	26,769	27,305	27,840
Upper 95% CI of the mean	33,947	33,386	34,096	34,806	35,516	36,227	36,937
Geometric mean	18,167	18,284	18,674	19,065	19,456	19,846	20,237

RBC=red blood cells

Table 9. No. of patients with underestimated and overestimated parasite densities calculated by different assumed RBC counts from $4.7x10^6$-$5.2x10^6/\mu L$, with the absolute RBC count as the standard in male patients (N=280)

Assumed RBC x10^6/µL	**No. of patients with underestimated parasite density**	**No. of patients with overestimated parasite density**	**P-value**
4.7	157	123	0.126
4.8	140	139	0.608
4.9	127	151	0.008
5.0	110	170	<0.001
5.1	90	187	<0.001
5.2	74	204	<0.001

RBC=red blood cells

Table 10. Parasite density calculated with absolute and assumed RBC counts from $4.2x10^6$-$4.7x10^6$/µL in female patients (N=71)

Parameter	Absolute RBC/µL	RBCx10^6/µL					
		4.7	4.8	4.9	5.0	5.1	5.2
Minimum	2,160	4,700	4,800	4,900	5,000	5,100	5,200
25 Percentile	10,105	9,400	9,600	9,800	10,000	10,200	10,400
Median	17,430	18,800	19,200	19,600	20,000	20,400	20,800
75 Percentile	34,320	37,600	38,400	39,200	40,000	40,800	41,600
Maximum	386,780	390,100	398,400	406,700	415,000	423,300	431,600
Mean	29,687	29,274	29,897	30,520	31,143	31,766	32,389
Std. Deviation	36,211	34,947	35,691	36,434	37,178	37,922	38,665
Std. Error of Mean	2,164	2,089	2,133	2,177	2,222	2,266	2,311
Lower 95% CI of the mean	25,427	25,163	25,698	26,234	26,769	27,305	27,840
Upper 95% CI of the mean	33,947	33,386	34,096	34,806	35,516	36,227	36,937
Geometric mean	18,167	18,284	18,674	19,065	19,456	19,846	20,237

RBC=red blood cells

Table 11. Underestimated and overestimated parasite density produced with different assumed RBC counts from $4.2x10^6$-$4.7x10^6$ /µL, with the absolute red cell count as the standard in female patients (N=71)

Assumed RBC x10^6/µL	No. of patients with underestimated parasite density	No. of patients with overestimated parasite density	P-value
4.2	42	28	0.409
4.3	38	33	0.977
4.4	37	34	0.395
4.5	32	38	0.062
4.6	25	46	0.002
4.7	19	52	<0.001

RBC=red blood cells