Original article

The reliability of blood film examination for malaria at the peripheral health unit

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Abstract

Background: Malaria is a common and serious problem in Ethiopia. Blood film examination is the best tool for diagnosing malaria where feasible.

Objective: To assess the reliability of blood film examination at the primary health care level.

Method: Two specimens were taken from all suspected patients in five health center and one hospital in north Gondar zone in Ethiopia. One to be stained and read by the *operational readers*, the other to be sent unstained to the reference reader.

Result: Out of 3625 patients whose blood film was sent, 44% were females and 28% were positive for malaria. The peak age was 15-29. The proportion of *P. falciparum* and *P. vivax* was 64.6% and 35.6%. The specificity and positive predictive values were low and the overall chance corrected agreement (kappa score) of the operational and reference reader was less than 0.53.

Conclusion and recommendation: The agreement and species identification of the operational and reference readers were low. Continuous retraining and supervision are indispensable. [*Ethiop.J.Health Dev.* 2003;17(3):197-204]

Background

Malaria has been recognized as causing a lot of human calamities (1). More than 65% of the population of Ethiopia living in 75% of the country is at risk of malaria (2). Gondar is one of the malaria risk areas in the country most of the district's population is at risk. Malaria undermines the health and welfare of families, endangers the survival and education of children, debilitates the active population and impoverishes individuals and countries (3, 4). There were repeated epidemics in the Ethiopia. The 1958 malaria epidemic caused around 3,000,000 cases and 150, 000 deaths have been reported, most of it in the Northwestern part of the country (5,6).

Clinical assessment is the approach to the diagnosis of malaria in remote areas with less facilities (7), especially during the epidemic season. Delegating management of febrile cases to be treated as malaria by less trained persons, in areas where trained health worker is not available is appropriate (2-4,7,8).

There are laboratory techniques that are using antigens of the malaria parasite as reagent. These are highly sensitive specific and with high predictive value. Such tests are used in some countries in Africa and other parts of the world, but are not widely available, since they are expensive (9,10).

However, in unstable malaria where there is seasonal variation, presumptive treatment of uncomplicated fever with anti-malarial drugs may result in potentially high proportion of misdiagnosis and consequent mismanagement during the low malaria transmission seasons (3,7).

In addition, when the recommended treatment for *P. falciparum* and *P. vivax* is different, blood film is very important to differentiate the species and give appropriate treatment to patients (4). There are at least 7 centres in

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Gondar Administrative Zone where blood film examination facilities are routinely available. We have observed that many patients are being managed as malaria cases in spite of a negative blood film result (11). That may be due to over suspiciousness of health workers or the perceived poor quality of laboratory findings. Therefore, the purpose of the study was to see the reliability of the blood film reading at the primary level and recommend a possible solution for identified deficiencies.

Methods

Study area: The study was conducted in North Gondar Administrative Zone. North Gondar is one of the zones in Amhara Regional State with an area of 53176 Km^2, divided into 18 Woredas and 546 Kebeles. It has more than 2.8 million population. The area exposed to malaria is 75%, number of malarious Kebeles are 346; population at risk is 64 %. The health service geographical coverage is around 35%; malaria was among the 10 top diseases in the outpatient departments of the health institutions in the zone in 2001-2002.

All the health centres and the two hospitals in the Zone have the facility to routinely do blood film. Among these health institutions, one hospital and five health centers were selected, based on accessibility and patient load. These are Gondar, Tseda, Kolladebe, Dabat and Aykel Health Centres, and GCMS hospital. Because the areas are known for epidemic malaria, the specimens were taken at two non-malaria epidemic seasons (January and August) and two malaria epidemic seasons (May- June and September – October).

Specimen collection: Patients from the out-patient departments of the health institutions were sent for blood film examination.

In each health institutions, 2 specimens, were taken from all suspected patients. One to be stained and read by the laboratory technician at the respective health institution (operational readers) and the other to be sent unstained. All positive and negative slides, or if the negative slides are more than 1.5 times the positive slides, 1.5 times the positive slides at each health institution in the particular month seen by the operational readers were collected, coded and given to the reference readers who were blinded to the readings of the operational readers. The readings of the technicians were documented in a form prepared for the survey. Unstained blood films prepared by the operational readers were also sent to be stained and read by the reference readers. A total of 1000 stained slides positive for malaria and another1355 negative slides were selected. The negative slides were selected randomly from the list of negative. The negative and positive slides were mixed and given to the reference readers for re-examination. A total of 4710 slides (2355 slides read by operational readers and 2355 unstained slides) were read and examined for malaria parasites by the reference readers.

The reference readers examined and returned the results of 2345 of the slides read by the operational readers, and 2332 of the slides they stained and read. Ten (0.42%) slides from the stained ones and 23(0.98%) of unstained were lost.

The staining technique used by both the operational readers and reference readers was Giemsa staining method (12,13).

Analysis: All the findings including the demographic data were documented on the prepared form. Analysis was made using Epi Info 2000 statistical package. Proportions and percentages were compared, and positive predictive value (PPV), negative predictive value (NPV), specificity, sensitivity, uncorrected and chance corrected agreement (Kappa score) were calculated.

Operational Definitions

Operational reader: is a laboratory worker who is either a malaria microscopist who has worked for more than 10 years or formally trained after high school as laboratory technician, awarded a diploma and working

mainly in the peripheral health centers, at least for a year.

Reference reader: is a laboratory technologist with bachelor degree or equivalent, worked for more than 15 years, and assigned at Gondar College of Medical Sciences as instructor in the field during the study period.

Reference Reader1= The reference reader rereads the slide prepared, stained and read by operational reader.
Reference Reader2= The reference reader reads the slides stained by the reference readers themselves.

Results

During the four-month study period there were 3625 patients whose blood film was sent for malaria diagnosis in the study health institutions. Among these, 1000 (28%) were positive for malaria. The positivity rate varies from 7% to 49% in the different health institutions. Of the patients who gave blood specimen 1595(44%) were females the rest were males (Table 1).

The age and sex distribution of patients whose slides were read were slightly different from the total population. Many of the patients were in the age group below five and between 15-29 years (Table 2). The positivity rate varies with seasons and between health institutions. It was high during the malaria epidemic season and low in the non-epidemic season.

Comparison was made between results obtained from operational readers and the reference reader. From slides stained and read by operational readers initially, overall specificity and positive predictive value are 73.7% and 58.1 % respectively. Agreement between the operational readers and the reference was 75%. But the chance corrected agreement or kappa score was 0.47 (Table 3).

Results of slides prepared by operational readers and stained and read by reference readers compared with those of operational readers' of the same patients, show the uncorrected agreement was 77% and the chance corrected agreement was 0.53 (Table 3).

Table 1: **Reference population by sex, and study period, in selected health institutions, North Gondar, 2001.**

Health institution	January		May-June		August		Sept.-Oct.		Sub total		Total			
	M	F	M	F	M	F	M	F	M	F	No	%	No pos	% pos
GCMS Hosp*	90	90	57	61	81	73	80	81	308	305	613	17	44	7
GONDAR	105	95	103	77	112	90	109	91	429	353	782	22	127	16
TSEDA	56	51	121	79	0	0	194	126	371	256	627	17	307	49
KOLADUBA	111	119	52	66	135	90	170	207	468	482	950	26	322	34
CHILGA	47	36	54	28	141	33	88	25	330	122	452	12	165	37
DABAT	13	17	32	14	19	16	60	31	124	77	201	6	35	17
Total	422	408	419	325	488	301	701	561	2030	1595	3625	100	1000	28
Sex %	43	57	60	40	56	44	65	35	60	40				

*GCMS Hosp = Gondar College of Medical Sciences Hospital

Table 2: **Age and sex distribution of the study population, North Gondar, 2001**.

Age group	Sex				Total	
	Female		Male			
	No	%	No	%	No	%
0-5	154	44	199	56	353	15
6-9	58	40	88	60	146	6.2
10-14	85	42.5	115	57.5	200	8.5
15-19	176	43	229	57	405	17.2
20-29	221	36	392	64	613	26.0
30-39	153	49	161	51	314	13.3
40-49	53	34	101	66	154	6.5
50-64	42	42	59	58	101	4.3
65+	9	29	22	71	31	1.3
Unknown age	17	68	8	32	25	1.1
Unknown sex					13	0.6
Grand total	968	41	1374	59	2355	100

Table 3: **Agreement of readers on detecting malaria parasites in selected North Gondar-zone health institutions based on slides stained at the peripheral health institution, 2001**

OR*	Malaria Parasites								
	RRI**								
	Positive	Negative	Total	Sensitivity	Specificity	PPV****	NPV*****	Agreement	Kappa
Positive	579	417	996	78.5%	73.7%	58.1%	88%	75%	0.47
Negative	159	1166	1325						
Total	738	1583	2321						
OR		RR2							
Positive	718	273	991	73.6%	79.5%	72.5%	80.4%	77%	0.53
Negative	258	1058	1316						
Total	976	1331	2307						

*OR = Operational Reader **RR1 = Reference Reader1 ***RR2 = Reference Reader2
****PPV = Positive Predictive Value *****NPV = Negative Predictive Value

Of the 1000 positive for malaria the identified species were *P. vivax* 356 (35.6 %) and *P. alciparum* 644 (64.4 %). Of all the positive 390(39%) were females 608 (60.8 %) were males and 2(0.2%) were unknown sex.

Observation was also made on agreement of species identification on slides stained by operational readers, the uncorrected agreement was 69%, kappa score 0.41. When the reading of operational readers was compared with the reading of the reference readers as the slide, which the references readers stained, the agreement was 70% with kappa score of 0.51 (Table 4).

Table 4: **Agreement of readers on identifying malaria species at the study sites staining done separately, North Gondar, 2001**

	Ref. Reader1					
Op. reader	Negative	*P. falciparum*	P.Vivax	Total	Agreement	Kappa
Negative	1166	140	19	1325	69%	0.41
P. falciparum	254	350	37	641		
P.vivax	163	106	86	355		
Total	1583	596	144	2321		
			RR2			
Op. reader	Negative	P.falciparum	P. vivax	Total	Agreement	Kappa
Negative	1058	218	40	1316	70%	0.51
P.fa;co[ari,	168	442	28	638		
P.vivax	105	126	122	353		
Total	1331	786	190	2307		

OR = Op. reader = Operational Reader RR1 = Ref. reader1 = Reference Reader1 RR2 = Reference Reader2

When we see the specificity and positive predictive value of the test at each health institution it was least at Aykel Health Centre (64%, 38%) borderline at Gondar Health Centre (69%, 37%) and better at GCMS (93%, 60%). The sensitivity and negative predictive value of this test was least at Tseda health Centre 73.3 and 78% respectively (Table 5).

Table 5: **Blood film reading agreement of laboratory technicians for malaria parasite, by health institution North Gondar, 2001**

Health Institution	Op. reader	Ref. Reader1			Sensitivity %	Specificity %	PPV %	NPV %	Agreement %	Kappa
		Positive	Negative	Total						
GCMS	Positive	26	17	43	89.6	93	60	99	93	0.70
	Negative	3	228	231						
	Total	29	245	274						
Gondar	Positive	47	80	127	67.1	37	69	89	69	0.38
	Negative	23	181	204						
	Total	70	261	331						
Tseda	Positive	238	69	307	77.3	78	78	78	78	0.56
	Negative	70	243	313						
	Total	308	312	620						
Kolladeba	Positive	186	135	321	93.4	65	58	87	72	0.45
	Negative	37	248	285						
	Total	223	383	606						
Aykel	Positive	62	101	163	70.5	64	38	87	66	0.28
	Negative	26	181	207						
	Total	88	282	370						
Dabat	Positive	20	15	35	87.0	88	57	97	87	0.61
	Negative	3	105	108						
	Total	23	120	143						

Discussion

The positivity rate for malaria parasite varies among different health institutions. In some of them it is as low as 7%, in others as high as 49%. This variability is mainly related to the variability in the prevalence of malaria in different areas of the zone. The skills of the different health workers may also contribute for this variation.

As observed, in our findings, the specificity and positive predictive value of the test is not-satisfactory (though it is better in some than others). This implies that there are fairly good number of false positive patients (as high as 63 % of the positive) who are exposed to unnecessary treatment.

But the most important and worrisome findings are the low sensitivity and negative predictive value of this test at various health institutions. This tells us there were many false negative cases (as high as 22% of negatives), whose correct diagnosis was missed that might lead to delayed treatment for malaria, development of serious complications and death or exposure to unnecessary treatment with other drugs (4,15).

The overall chance corrected agreement between the operational and the reference readers were found to be low, it would have been better had it been at least 0.70(15). In fact much lower than the cross checking result of the slides from the sector malaria laboratories by the head quarter laboratory in the 70s. At that time the Kappa score, PPV, and NPV were 0.97, 99% and 97%, respectively (16).

In areas where there is unstable malaria, the need for blood film in severe and complicated cases is obvious. Moreover, this investigation has a significant role in the diagnosis of uncomplicated malaria, especially to confirm epidemics, diagnose sporadic cases during non-epidemic seasons, when *P. vivax* is a major problem and the recommended treatment is different from that of *P. falciparum*, and last but not least when resistance is suspected (2,4,14). However, if the test done is not fairly reproducible, it is difficult to rely on it. Therefore, physicians will continue to treat based on clinical impression despite the laboratory result (11).

Hence, improving the skills of laboratory workers to detect malaria parasites is an urgent need. Enhancing the agreement level and the reliability of the technicians is desirable and achievable. Slides from the peripheral laboratories where diagnosis of malaria is made should be sent to a reference centre for verification and quality control. Central laboratories should participate in this type of relevant quality assurance scheme (10,17-19). Refresher courses, workshops and seminars in the field of laboratory technology are lacking in the Region (20).

Conclusion and recommendations: The agreements of readings of the blood film specimens by peripheral and reference technicians were very low. For maintaining and raising the skill of the laboratory technicians continuous retraining and supervision, with quality control schemes are indispensable.

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