Modified Giemsa Staining for Rapid Diagnosis of Malaria Infection

J. Iqbal^a P.R. Hira^a F. Al-Ali^b N. Khalid^a A. Sher^c

^aDepartment of Microbiology, Faculty of Medicine, Kuwait University, and ^bDepartment of Laboratories, District General Hospital, Farwaniya, and ^cMalaria Laboratory, Ministry of Health, Kuwait

Key Words

Malaria · Giemsa staining · Microscopy

Abstract

Objectives: To develop and evaluate a rapid method for the diagnosis of malaria infection by microscopy of stained blood films. **Subjects and Methods:** Blood specimens were collected from randomly selected confirmed malaria cases (n = 75) and suspected malaria cases (n = 175). The microscopy was done on each set of blood films stained by modified and the standard Giemsa staining methods. **Results:** All the 75 previously diagnosed malaria cases were confirmed by the microscopy of blood films stained by both methods. Forty-nine (28%) of the 175 cases suspected for malaria infection showed malarial parasites on microscopy of blood films stained by both methods. However, due to homogeneous staining and clearer background of the blood films it was possible to determine the parasite species in 65% of the cases on microscopy of the thick films stained with the modified method compared to only 20% with the standard method. Further, the turnaround time for reporting the microscopy test result was 15–20 and 45–50 min with modified and standard staining methods, respectively. **Conclusion:** Our data showed that performance of the modified staining method in detecting malarial parasites was comparable to that of the standard staining method. Moreover, the modified staining method was rapid, easy to use, and reliable.

Introduction

Malaria causes significant morbidity and mortality worldwide, even in countries with imported malaria [1]. As such the global resurgence of malaria has become a health problem not only in malaria-endemic countries but also in those with imported malaria such as Kuwait [2]. The biological diagnosis of malaria is urgent to avoid rapid and fatal outcome. Microscopy of standard Giemsa-stained thick and thin blood film still remains the current gold standard for diagnosis. However, its performance is linked to technical expertise of the microscopist and is time-consuming [3]. Recently alternative rapid nonmicroscopic methods that include acridine orange-stained parasites in capillary centrifuged blood, rapid immunochromatographic assays and the sensitive molecular techniques such as DNA hybridization and polymerase chain reaction have been introduced to overcome the limitations of conventional microscopy [3–6]. However, these methods have their own limitations and thus should be regarded as complementary methods to the traditional diagnosis by microscopy. Therefore there is a need to develop another diagnostic method with less limitations.

In this study, we describe a modification of the traditional Giemsa staining method and compared both methods for identification of malarial parasites, turnaround time for reporting and its application in the routine clinical microbiology laboratory.

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Jamshaid Iqbal
Department of Microbiology
Faculty of Medicine, Kuwait University
PO Box 24923, 13110 Safat (Kuwait)
Tel. +965 5319602, Fax +965 5332719, E-Mail iqbal@hsc.kuniv.edu.kw

Table 1. Microscopy of stained Giemsa-stained blood films for the detection of malarial parasite species^a

Study groups	Modified Giemsa staining			Standard Giemsa staining		
	<i>P. vivax</i>	<i>P. falciparum</i>	mixed	<i>P. vivax</i>	<i>P. falciparum</i>	mixed
Malaria patients	49	21	5	49	21	5
Suspected patients	39	7	3 ^b	41 ^b	7	1

^a One of the microscopists failed to detect 2 patients with *P. falciparum* and 1 with mixed infection. The blood films were reexamined to confirm the diagnosis.

^b Two additional cases of mixed (*P. falciparum* and *P. vivax*) infections were detected when the blood films were stained by the modified staining method. Both these cases had low parasitemia (<50 parasites per 200 leukocytes).

Subjects and Methods

Subjects

A total of 250 randomly selected patients were enrolled in the study; 75 of them were diagnosed with malaria and 175 were suspected malaria cases. The study was carried out during 1997–1998. The study was reviewed and approved by the Ethics Committee, Faculty of Medicine, Kuwait University. An authorized consent was obtained from each individual.

Malaria Patients

These individuals presented at the Malaria Laboratory and Infectious Diseases Hospital, Kuwait with fever. Seventy-two of the 75 patients were migrant workers from malaria-endemic countries in Asia or Africa; 2 were Europeans and 1 Kuwaiti with a recent history of travel to a malaria-endemic country. The diagnosis of malaria infection was confirmed by the presence of malarial parasites in blood films stained with the standard Giemsa staining method.

Suspected Malaria Cases

These individuals presented with fever at various Health Centers of the Ministry of Health, Kuwait and had a recent history of visit to a malaria-endemic area. From each patient 0.5 ml of blood was collected in EDTA-coated vacutainers and two sets of thick and thin blood films were made immediately. The thick and thin blood films were made on the same slide. The blood films were labeled and coded. One set of the blood films was stained with the modified Giemsa staining method and the second set was stained with the standard Giemsa staining method as described below.

Giemsa Stain

A 10% Giemsa stain was prepared by taking 10 ml of Giemsa stain (Fluka Chemicals, No. 48900, Switzerland) in 90 ml of phosphate buffer (pH 7.2) and filtering the stain. A fresh stain was made every 3rd day.

Modified Giemsa Staining

A thick blood film was made on one third of the slide, and a thin blood film was spread on the rest of the same slide. The blood films were air-dried for 2–3 min. The thick blood film was fixed by dipping briefly 3 times in acetone in a Coplin jar and the thin film was fixed in methanol (20–30 s). Immediately after fixation the slides were placed in the filtered Giemsa stain for 10 min. The slides were then

washed by three gentle dips in phosphate buffer (pH 7.2) and placed upright to air-dry.

Standard Giemsa Method

The blood films were made, air-dried and stained by the standard Giemsa staining method [6–8]. Briefly, both thick and thin blood films were made on the same slide and air-dried for 2–3 min. The blood films were then stained with 5% Giemsa stain for 30 min.

Examination of Slides

Three experienced microscopists examined the stained blood films blindly. In case of any discrepancy among the microscopists in detection of parasites, the blood film was reexamined by all the 3 microscopists to reach a consensus. The blood films were also examined for: staining characteristics of the film as a whole and of malarial parasites in particular, washing off of the films; ease of differentiation of different parasite stages and species, and the turnaround time for reporting the results. In addition to screening for malarial parasites, the microscopists were asked to evaluate each blood film for its staining pattern, speed and ease of reading the blood films. The parasite density in the thick blood films was calculated by counting the number of parasites per 200 white blood cells.

Results

All the 75 malaria cases were confirmed by microscopic examination of blood films stained by both modified as well as standard staining methods. Twenty-one patients (28%) had *Plasmodium falciparum*, 49 (65%) had *Plasmodium vivax* and 5 had mixed (*P. falciparum* and *P. vivax*) parasites. The results are shown in table 1. The parasite density ranged between 50 and 1,250 parasites per 200 leukocytes. Twenty-three of the 75 cases had parasitemia lower than 75 parasites per 200 leukocytes and only 9 cases had parasitemia higher than 250 parasites per 200 leukocytes. Forty-nine of the 175 (28%) cases in the suspected malaria patients showed malarial parasites on microscopy by both staining methods. The microscopy of

Table 2. Evaluation of performance of modified and standard Giemsa staining techniques for the detection of malarial parasite by microscopy¹

Parameters	Modified Giemsa staining	Standard Giemsa staining
<i>Technical</i>		
Turnaround time, min	15–20	45–60
Washing off the film	partial, occasional (2%)	nil
Staining	homogeneous occasionally heterogeneous	homogeneous occasionally heterogeneous
<i>Staining characteristics</i>		
Red blood cell staining		
Thick film	complete hemolysis	incomplete hemolysis
Thin film	homogeneous staining	homogeneous staining
<i>Parasite staining</i>		
Parasite staining		
Thick/thin	parasite/malarial pigment distinct	parasite/malarial pigment distinct
Species identification		
Thick film	possible (65% cases)	less possible (20% cases)
Thin film	homogeneous staining	homogeneous staining
<i>Acceptance by technical staff</i> ²	highly acceptable	satisfactory

¹ The microscopists observed minor variations in the reported staining characteristics with the modified staining method. However, there was a general agreement among all the 3 microscopists on most of the staining characteristics reported.

² It was evaluated by noting technical staff preference to use modified staining method over the standard method.

blood films stained by the modified staining method showed that 39 (22.3%) patients had *P. vivax* parasites, 7 (4%) had *P. falciparum* and 3 patients had mixed (*P. falciparum* and *P. vivax*) parasites (table 1). However, the microscopy of blood films stained by the standard staining method detected *P. vivax* parasites in 41 (23.4%) patients, *P. falciparum* in 7 (4%) and mixed (*P. falciparum* and *P. vivax*) parasites in 1 patient (table 1). One of the microscopists failed to detect 2 patients with *P. falciparum* and 1 with mixed infection.

Table 2 shows performance of both the techniques in terms of total time involved, ease of use, and reliability. With the modified staining method, the staining of both thick and thin blood films was more homogeneous, background clearer and thus, the identification of different species and stages of the parasite was relatively easier and quicker than with the standard staining method. In the positive cases, 65% of the parasite species could be determined on thick blood film microscopy of the modified method compared to 20% with the standard staining technique. However, partial washoff of the blood film was observed relatively more often in blood films stained by the modified method than in those with the standard

method. The turnaround time, i.e. the time taken from receiving a specimen to reporting of results, was approximately 15–20 min for the modified staining method compared to 45–50 min by the standard staining method.

Discussion

Both techniques were equally able to detect malarial parasites in the blood films. All previously diagnosed malaria cases were confirmed and species determined by the modified staining technique. Traditionally, the stained thick blood films allow only the screening of films for the presence or absence of parasite in the specimen. The parasite species is determined by examination of thin blood films. However, in the modified staining method, distinct staining of malarial parasite and malarial pigment and clear background allowed determination of parasite species even in the thick blood films.

In our opinion the improved performance of the modified technique is primarily due to the fixation of both thick and thin blood films in acetone and methanol,

respectively, before staining. Using acetone to fix the thick films has many advantages:

(a) It removes water from the plasma proteins that causes proteins to precipitate and adhere to the surface of microscopic slides, minimizing the chances of washing off the blood films during staining. Furthermore, making a thin or thick film on a low-hemoglobin (Hb) blood is always problematic as the blood film flakes off much more easily during the washing procedure. Several of our patients had Hb <9 g/dl, but none of their blood smears flaked off after fixing with acetone.

(b) It makes the cell membranes of both red and white blood cells more porous by removing water from the cell membrane and releasing Hb thereby making it easier to stain the parasites. The distinctive features of various species of the malaria parasites were easily recognized and thus with experience, even a thick film could be used for species differentiation by an experienced microscopist. Thus, the modified staining method not only reduced the time for staining to 10 min but also reduced the time for microscopic examination due to clearer background and homogeneous staining. The parasite species are normally determined in thick film where the specimen was concentrated. The distinct staining of malarial parasite against a clear background was due to the additional fixation steps in the modified staining method that made the cell membranes more porous and thus released the Hb out of the cells thereby making it easier to stain and visualize the parasites.

Occasional variability in the intensity of staining of blood film with the modified staining method was noticed. This was probably due to the uneven thickness of the thick blood film during preparation. However, there was no problem in detecting malarial parasites and identification of the parasite species due to presence of properly

stained organisms in other parts of the film. The other advantage of having both thick and thin blood films on the same slide is the ease with which the slides can be examined without the loss or deferred examination of either film.

In nonendemic countries such as Kuwait, with peculiar requirements for the treatment and management of malaria, rapid diagnosis of malaria infection is utmost important to reduce the morbidity and mortality. However, we believe that this method, which requires no special equipment, is easily applicable even in developing endemic countries. The only caveat is that in many developing countries the blood for malaria examination is done from a finger-prick sample of blood. This, in fact, is better as there are less problems with the presence of fibrins, which may infect as glue and thereby further attach the thick film to the glass slide.

Conclusion

In terms of performance, ease of use and reliability, the modified Giemsa-staining method is better than the traditional Giemsa-staining method for microscopical diagnosis of malaria. The turnaround time for reporting the test results was reduced to 15–20 min. Furthermore, it could be an appropriate method for both clinical and epidemiological studies.

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