Comparing the Buffy Coat and Traditional Blood Smears in the Microscopic Diagnosis of Malaria

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ABSTRACT

The purpose of this study was to compare buffy coat smear (BCS) and traditional blood smear (TBS) in the microscopic diagnosis of malaria using Giemsa-stained blood films. Blood samples were collected from 130 patients clinically suspected of malaria. Thin and thick blood films were prepared using whole blood and buffy coat for each sample. The Giemsa-stained slides were examined to determine the number of parasites per high power field (HPF). Data were analysed using the student T test, Chi-square and correlation analysis. Eleven (8.5%; CI: 3.7 – 13.3) specimen were positive for the TBS and 16 (12.3%; CI: 6.7 – 18) were positive for the BCS including all the specimens that were positive with the TBS. The sensitivity and specificity of the BCS test were 100 % and 95.8 % respectively. The mean parasite per HPF was significantly higher for the BCS compared to the TBS (p = 0.0352). BCS was observed to concentrate the parasites 3.24 times more than TBS. A strong correlation was observed between the TBS and BCS (r = 0.844, p = 0.0011). Malaria parasites were detected in five (3.85 %) specimens using the BCS which were missed with the TBS. Microfilariae of Loa loa were also detected in one of the BSC specimen.

This study clearly demonstrates that examination of Giemsa-stained films of buffy coat will result in a higher sensitivity than TBS, without any additional requirement of reagents and equipment, and using basically the same staining protocol. It is therefore recommended in developing countries endemic for malaria.

Keywords: Buffy coat smear, traditional blood smear, Giemsa, microscopy, malaria diagnosis, Hospital


INTRODUCTION

Malaria is one of the most important parasitic infection today. The disease is widespread in tropical and subtropical regions around the equator, covering much of Sub-Saharan Africa, Asia and Latin America (Caraballo and Kevin 2014), which largely encompasses the developing countries of the world. An estimated 219 million cases of malaria were reported in 2012, resulting in 660,000 deaths (Nadjmand Behrens, 2012; WHO, 2012), most deaths occurring in children below 15years in Sub-Saharan Africa (Murray et al, 2012). Five species of Plasmodium cause malaria in humans including P. vivax, P. ovale, P. malariae, P. Knowlesi and P. falciparum, with the latter being the most virulent, accounting for almost all deaths caused by malaria (Olupot-Olupot and Maitland, 2013). Examination of Giemsa-stained blood films with light microscopy is considered the gold standard for malaria diagnosis (Milne et al, 1994; Warhurst and Williams, 1996; Kilian et al, 2000; Coleman et al, 2006; Wongsrichanalai, 2007). This is
the most technique widely used in the world today (Wilson, 2012). In expert hands, the method can be very sensitive and detect parasite as low as < 5 parasite/µl (Moody, 2012). Many of the deaths due to malaria could be prevented if the disease is well managed. Improvement in management also entails improvement in the current diagnostic techniques. There has been a considerable improvement in the diagnosis of malaria with the development of rapid diagnostic tests (Murray et al. 2008 and Moody, 2012). Other diagnostic techniques exist including the quantitative buffy coat (QBC), enzyme linked immunoassays (ELISA), and polymerase chain reaction (PCR), but these tests are expensive for resource limited settings as seen in most developing countries. Light microscopic examination of Giemsa stained films is still the most widely used technique. Although light microscopic examination of Giemsa-stained films is relatively cheap, the technique is labour intensive, time-consuming, and requires substantial training and expertise (Murray et al, 2008), hence the need for improvement. An earlier study comparing capillary and venous blood had demonstrated that capillary blood was more sensitive than venous blood in the detection of malaria parasite in Giemsa-stained films (Njunda et al, 2013).

It was in this light that we decided we decided to compare a technique using the buffy coat to the traditional blood smears (using whole blood) in the microscopic diagnosis of malaria. This technique that uses the buffy coat is unlike the QBC technique which requires a dye (acridine orange) in addition to some very expensive instrumentation like epifluorescent microscope.

MATERIALS AND METHODS

Study design and setting

This study was a comparative study. The study was carried out at the University Teaching Hospital (French: Centre Hospitalier et Universitaire de Yaoundé – CHU). The hospital was designed for training medical doctors. The hospital is situated in Yaounde (3°52’N 11°31’E), the capital of Cameroon. Yaounde has a population of approximately 2.5 million, making it the second largest city in Cameroon after Douala. Yaoundé is a very diverse city with people from different works of life and is home to most of the administrative structures in the country.

Study population

Patients clinically suspected of malaria were enrolled. Males and females of all ages were eligible to participate in the study. The participants were required to provide their signed informed consent of which the study protocol was explained to them in English or French. For children and minors, their parents or guardians signed the informed consent form on their behalf. Excluded from the study were patients who were on or had been on antimalarial medication for within the past one week prior to the study. Patients who refused to give their signed informed consent were also excluded from the study. The study protocol was approved by the Faculty of Health Sciences Institutional review Board (FHS IRB) of the University of Buea, Cameroon.

Sample collection and processing

About 3 – 4 ml of whole blood was collected into citrated test tubes respecting aseptic techniques from consented participants and processed as follows:

To prepare blood films using the traditional methods, about 10 µl of whole blood was placed on a clean grease-free microscope glass slide at two spots to prepare a thick and thin blood films. The thin film was fixed with 95 % methanol for 2 mins and the films stained with 10 % Giemsa (diluted 1 in 10) for 25 – 30 mins.

To prepare the buffy coat films, the citrate anticoagulated blood was centrifuged at 3000 g for 5 mins. The plasma was pipetted out, up to the interphase between the plasma and red cells. The buffy coat and the red cell layer just below it were aspirated using a micropipette and transferred into a test tube and mixed thoroughly by aspirating and dispensing several times to attain a homogenous mixture. Thin and thick films were then prepared and stained as mentioned above.

The blood films were then rinsed and allowed to air dry prior to examination with a microscope. The slides were examined by two expert microscopists who were blinded to the results of the other. For each slide a preliminary screening was done using the 40X objective to enable the selection of the best areas in terms of staining and any other peculiarities. The thick films were observed first followed by the thin films using the 100X objective. The number of parasites per HPF were gotten by counting the number of parasite per HPF until 20 fields were reached and dividing the number by 20 for all the specimens.
Table 1. Sensitivity and specificity of the BCS using TBS as the gold.

<table>
<thead>
<tr>
<th></th>
<th>TBS positive</th>
<th>TBS negative</th>
<th>Total</th>
<th>Predictive value ( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS positive</td>
<td>11</td>
<td>5</td>
<td>16</td>
<td>68.75</td>
</tr>
<tr>
<td>BSC negative</td>
<td>0</td>
<td>114</td>
<td>114</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>119</td>
<td>130</td>
<td></td>
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<tr>
<td>Sensitivity ( %)</td>
<td></td>
<td></td>
<td>100</td>
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<tr>
<td>Specificity ( %)</td>
<td></td>
<td></td>
<td>95.8</td>
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$X^2$ (Yates corrected) = 76.97, p < 0.001

Statistical analysis

Data were entered into Microsoft Excel spreadsheet and analysed using Stata® version 12.1 (StataCorp LP). Tests performed included the student T test, Chi-square and Correlation analysis. Statistical significance was set at $p < 0.005$.

RESULTS

One hundred and thirty (130) participants were successfully enrolled in the study including 84 (64.6 %) females and 46 (35.4 %) males. The ages of the participants ranged between 5 and 85 (mean ± SD = 26.23±19.73).

Among the 130 specimens that were examined, 114 (87.7 %) were negative for both TBS and BCS, 11 (8.5 %; CI: 3.7 – 13.3) were positive for the TBS and 16 (12.3 %; CI: 6.7 – 18) were positive for the BCS (Figure 1). Microfilariae of *Loa loa* were observed in one of the BCS specimen and not in the corresponding TBS specimen.

The sensitivity and specificity of BCS using TBS as the gold were 100 % and 95.8 % respectively (Table 1).

For all the samples examined, the parasite per HPF for TBS ranged from 0.75 to 53 parasites (mean±SD = 18.02±22.17) meanwhile the parasite per HPF for the BCS ranged from 0.167 to 271 parasites (mean±SD = 40.1±73.44). The mean parasite per HPF were significantly higher in the BCS compared to the TBS ($p = 0.0352$). A strongly positive correlation was observed between the parasite per HPF obtain by TBS and BCS ($r = 0.844$, $p = 0.0011$) (Figure 2).
The difference in the number positive between the TBS (8.46%) and the BCS (12.31%) implies that using only the TBS, 3.85% of positive cases would have been missed.  
Comparison between the mean parasites per HPF for the TBS and BCS methods show that the BCS method was able to concentrate the parasites 3.24 times.

DISCUSSION

The purpose of this study was to compare the buffy coat and the traditional blood smears in the microscopic diagnosis of malaria using Giemsa-stained films.

In this study, it was observed that 11 (8.5%) of the 130 specimen were positive with the traditional blood smears prepared using whole blood meanwhile 16 (12.3%) were positive with the buffy coat prepared smears. The 16 specimen positive with the buffy coat smears included all those that were positive with the traditional blood smears, an indication that the diagnosis of malaria using buffy coat smears stained with Giemsa was more sensitive than using the traditional blood smears with whole blood. In this study we observed a sensitivity of 100 % for the buffy coat smears. Similar findings of the buffy coat smear having a superior sensitivity have also been reported by Bhandari et al, (2008). The improved sensitivity could be the result of the concentration of the parasite in the buffy coat during centrifugation. Malaria parasitized RBC are lighter or have a lower density that the unparasitized RBC, hence easily float and concentrate in or slightly below the buffy coat upon centrifugation. In this study it was observed that using the buffy coat was able to concentrate the malaria parasites by as much as 3.24 times that observed with the traditional blood smears. The improved sensitivity seen with the buffy coat smear have some important implications;

Firstly the improved sensitivity will lead to a reduction in the number of missed diagnosis. In this study, 5 (3.85 %) positive specimen that were missed with the traditional blood smear were easily detected with the buffy coat prepared smear. Another problem that has long been recognised with the Giemsa-stained blood films prepared with whole blood was the poor sensitivity when not in expert hands (Moody 2012). Using the buffy coat to prepare smear will solve some of the problems of shortages of expert microscopists trained in malaria microscopy often seen in developing countries in that the improved sensitivity will imply the malaria parasites will be easily detected upon concentration even by non-

**Figure 2.** Correlation analysis between TBS and BCS shows a strong positive association.
experts working in Medical diagnostic laboratories in malaria endemic areas.

Secondly, use of the buffy coat smear in diagnosis of malaria will have a major implication in the quantification of the parasites (parasitaemia). Physicians in malaria endemic areas routinely use the parasitaemia as one of the parameters to determine malaria severity and making decision as to whether it warrants admission or not, and also to make decision on the antimalarial treatment. Where the parasitaemia is very high is generally considered severe malaria and thus requires admission and intensive care. So by using the buffy coat knowing its concentration ability of the parasite will entails that the parasitaemia will be inflated in many cases which will have a direct effect on the management of the disease. In this study, it was observed that there was a strong correlation between the traditional blood smear and buffy coat parasitaemia (r = 0.844, p = 0.0011) which implies an increase in the parasitaemia seen in the traditional blood smears will lead to a proportionate increase in the parasitaemia in the buffy coat smear. But where the blood picture is required, the buffy coat smears may not be useful used since they are devoid of red blood cells.

Thirdly, using the buffy coat may be advantageous as the concentrating ability may also lead to the detection of other blood parasites like trypanosomes, and microfilariae that are also very prevalent in developing countries. In this study the parasite Loa loa was observed in one of the buffy coat smear.

The increased sensitivity in the detection of malaria parasite using the buffy coat seen here can be talked of as improvement at almost no cost. Unlike the quantitative buffy coat (QBC) technique that requires very expensive instrumentation in addition to fluorescent dye as mentioned above, this techniques is a very simple and inexpensive technique that requires staining with Giemsa like the traditional method involving whole blood except that it involves the use of the buffy coat.

The simple and inexpensive nature of this method implies it can easily be implemented in resource limited settings as seen in most developing countries endemic for malaria to improve on the diagnosis of malaria.

CONCLUSION

Microscopic examination of Giemsa-stained films prepared with the buffy coat was observed to have a sensitivity and specificity of 100 % and 95.8 % respectively. The buffy coat smear was observed to concentrate the malaria parasite 3.24 times more than the TBS, with an eventual increase in the sensitivity of detection of malaria parasites without any additional requirements of reagents and equipment; and using the same staining procedure as the traditional blood smears. With the buffy coat smear, other blood parasites including microfilariae can also be detected. Because of its simplicity and inexpensive nature, and better sensitivity, the buffy coat smear is therefore recommended for the diagnosis of malaria in resource limited settings as commonly seen in developing countries endemic for malaria.

Competing interest

The authors declare that they have no competing interest.

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REFERENCES


