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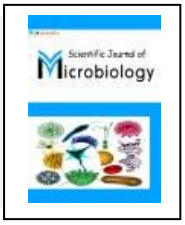

Original article

Comparison of capillary and venous blood using blood film microscopy in the detection of malaria parasites: A hospital based study

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ABSTRACT

Malaria mainly due to *Plasmodium falciparum* is associated with great morbidity and mortality in Sub-Saharan Africa especially in children under 5 years. In order to eradicate the disease and avoid complications that may arise from severe infection, there is need to improve in management which includes evaluation of the current diagnostic methods. Diagnosis of malaria in resource limited and developing countries is commonly done by the detection of blood stages of the Plasmodia in Giemsa stained blood films by light microscopy. Blood films are commonly prepared using capillary or venous blood. This study was aimed at comparing the sensitivity of capillary and venous blood in the detection of malaria parasites. Two blood films were prepared from the capillary and venous blood, air-dried, stained and examined following standard protocol by expert microscopists who were blinded from the results of the others. 150 participants including 95 (63.3%) women and 55 (36.7%) men were recruited for the study. The mean age of the participants was 30years (ranging from 7 to 66years). 44 (29.3%) of the 150 blood films prepared from capillary blood were found to be positive meanwhile 26 (17.3%) of the 150 venous blood films were found to be positive. The difference in the rate of malaria parasite detection in capillary blood smear was significantly higher than that with the venous blood (P=0.0109). No significant difference (P=0.8999) was observed between the capillary blood parasitaemia (3935parasites/mm³) and

venous blood parasitaemia (3407parasites/mm³). No significant correlation was observed between the capillary and venous blood parasitaemia (r = 0.3497, P = 0.1842). We came to the conclusion that usage of capillary blood to diagnose malaria was more sensitive than venous blood and these findings may impact routine clinical practice; hence improve on the management of malaria in endemic areas. We recommend that studies that employ light microscopy to detect malaria parasites including the annual figures posted by the WHO, should state whether it was capillary or venous blood that was used to obtain data as it is evident from this study that the prevalence of malaria is dependent on the source of peripheral blood.

1. Introduction

Malaria is a disease caused by protozoa of the genus *Plasmodium*. There are currently 5 known species that infect humans (*P. vivax*, *P. falciparum*, *P ovale*, *P malariae* and *P. knowlesi*) (Sarker et al., 2009; Collins, 2012). *Plasmodium falciparum* is the most common cause of malaria representing 90% of cases in Sub-Saharan Africa (Mendis et al., 2001). In Sub-Saharan Africa malaria is associated with great morbidity and mortality especially in children under 5years (Snow et al., 2005). Efforts are now base on the total eradication of malaria and to avoid its complications, through mass distribution of insect treated bed nets and improvement in the management of the disease. Improvement in management of malaria will entail evaluation of the current diagnostic techniques.

Examination of Giemsa stained blood films with light microscopy is considered the gold standard of diagnosis (Milne et al., 1994; Warhurst and Williams, 1996; Kilian et al., 2000; Coleman et al., 2006; Wangsrichanalai et al., 2007). With well prepared and properly stained blood films, microscopy is very sensitive (can detect parasite as low as 5-10 parasite/mm3) in expert hands, and also very specific (Moody, 2002). Blood films can be prepared using capillary and venous blood but the sensitivity of both capillary and venous blood in the detection of malaria has been a subject of debate.

This hospital based study was aimed at comparing the sensitivity of capillary and venous blood prepared films for the detection of malaria parasites from specimens collected form randomly selected patients suspected of malaria.

2. Materials and methods

2.1. Study site

This study was performed using the Laquintinie hospital (reference hospital) in Douala. Douala is the economic capital of Cameroon. With a population of 2,446,945, Douala is mainly a commercial area. Douala features a tropical monsoon climate, with relatively constant temperatures throughout the course of the year. The city typically features warm and humid conditions with an average annual temperature of 27.0 °C (80.6 °F) and an average humidity of 85% (WMO, 2012). Douala sees plentiful rainfall during the course of the year, experiencing on average roughly of 3,600mm (140 in) precipitation of rainfall per year (WMO, 2012).

2.2. Study population and ethical clearance

This study was approved by the Institutional Review Board (IRB) of the faculty of Health Sciences of the University of Buea, Buea, Cameroon. Participants were recruited from the outpatient department (OPD) of the Laquintinie hospital. Patients who presented with symptoms of malaria irrespective of age or sex were eligible for the study. Patients who were on antimalarial treatment within a month prior to the study were not eligible. Participants were required to give a written informed consent to the study which was duly explained to them in English, French and the local Pidgin English. A questionnaire was administered to consented participants in order to obtain information on the demographic distribution of participants. In the case of children and others who could

not provide their informed consent and take part in the questionnaire, their immediate carer, next of kin or parents were invited to do so.

2.3. Detection of malaria parasite

Two thick and thin blood films were prepared from every participants using both capillary and venous blood. Capillary blood was obtained using finger prick and venous blood was obtained from blood drawn into EDTA tubes. The blood films were air-dried and stained with 10% Giemsa (1 in 20 dilutions) for 25-30minutes.

Detection of malaria parasite was done using a light microscope by two expert microscopists, who were blinded to the results of the other. In case of any discrepancies in the result obtained by the two microscopists, a third microscopists (reference) was invited to confirm the results and the results obtained by the third microscopists were presumed conclusive, a method previously described by Noedl *et al.*[13]. The thick films were screened for 200 fields using the 100X (oil immersion) objective. If malaria parasites were seen, the thin film was then used to quantify parasites as well as determine the species of *Plasmodium*. The asexual stages of the *Plasmodium* were counted until 200 WBC were reached and the number obtained was divided by 200 and then multiplied by 8000 to give numbers in parasite per mm^3 [14].

2.4. Statistical analysis

Statistical analysis was performed using MedCal® version 12·2·1·0. Wilcoxon test for paired samples was used to compare means. Correlation analysis was performed to determine relationships. *P* values < 0·05 were considered to be statistically significant.

2.5. Limitations

Despite the fact that detection of malaria parasites in blood films have been shown to be highly sensitive, sensitivity varies. More advanced methods like PCR could not be used to confirm these findings because they are very expensive and will required experts and hi-tech facilities to perform these analyses, which is not readily available in developing countries.

3. Results

3.1. Study participants

150 persons consented to the study. The study participants were made up of 95 (63.3%) females and 55 (36.7%) males. The mean age of the participants was 30years (range: 7years to 66years).

3.2. Test results

44 (29.3%) of the 150 smears prepared from capillary blood were found to be positive meanwhile 26 (17.3%) of the 150 smears prepared form venous blood were positive. 28 of the 44 positive capillary blood smears were positive exclusively for capillary blood. 16 were positive for both capillary and venous blood. And 10 of the 26 positive venous blood smears were positive exclusively for the venous blood (Fig. 1). The difference between the prevalence of malaria in capillary and venous blood was 12% (29.3% - 17.3%). This difference was observed to be significantly statistically (P= 0.0109).

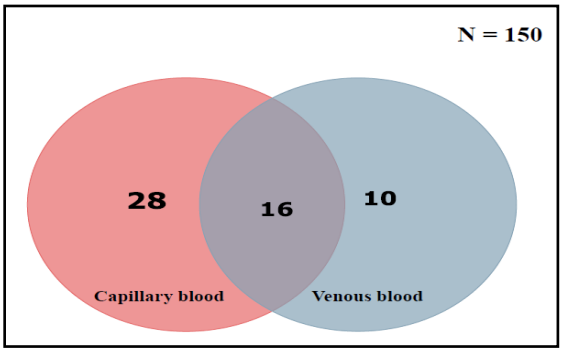

Fig. 1. Summary of result obtained from capillary and venous blood prepared smears.

The parasitaemia observed (Figure 2) was generally higher in specimen prepared from capillary blood (mean = 3935 parasite/mm³) than in specimen prepared from venous blood (mean = 3407 parasite/mm³), but the difference was not found to be significant statistically (P=0.8999).

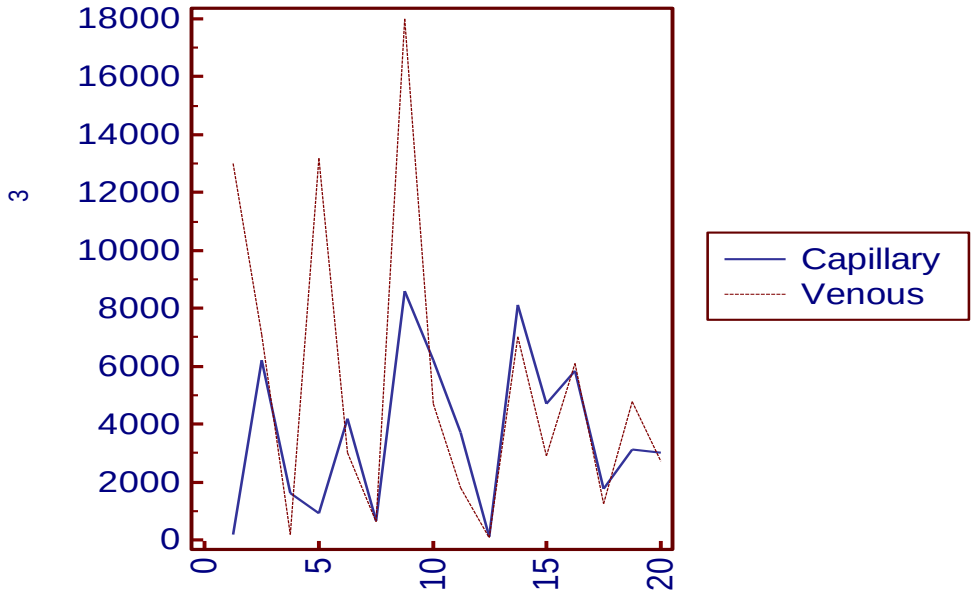

Fig. 2. Parasitaemia observed from capillary and venous blood specimen

In order to determine the relationship between capillary and venous blood prepared smear, correlation analysis was performed. No statistically significant correlation was observed (r = 0.3497, P = 0.1842) (Fig.3).

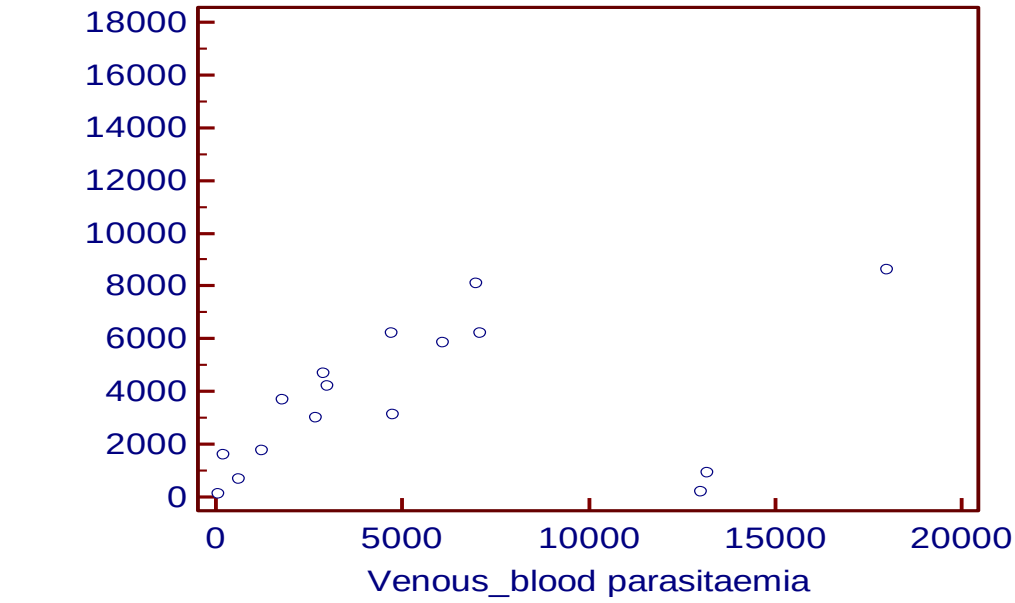

Fig. 3. Plot of capillary against venous blood parasitaemia.

4. Discussion

Malaria is one of the most important infectious diseases claiming lots of lives each year. The WHO estimates that in 2010, there were 219 million documented cases of malaria. That year, between 660,000 and 1.2 million people died from the disease (roughly 2000–3000 per day), many of whom were children in Africa (Nayyar et al., 2012). 90% of malaria cases in Sub-Saharan Africa are due to *Plasmodium falciparum* (Mendis et al., 2001). In developing countries with limited resources, malaria is commonly diagnosed by the detection of parasites in Giemsa stained blood films by light microscopy. This method is easy to perform and diagnosis can be achieved with great accuracy when performed by experts. However sensitivity varies greatly. The desire for a total eradication of malaria in endemic areas in order to avoid its complications has led to the evaluation of the different diagnostic techniques to improve on the management of the disease. There arises the need to optimize the diagnosis of malaria by light microscopy in these endemic areas. Blood films for detection of malaria parasites are commonly prepared from capillary and venous blood. The issue of the sensitivity of capillary and venous blood when use to prepare blood films therefore arises and so too the need for further investigation.

In this study, capillary and venous blood were used to detect malaria parasites from patients suspected of malaria using light microscopy by experts blinded to the results of the others. We observed that the number of positive cases with capillary blood (44 of 150) was significantly (P= 0.0109) higher than the number of positive cases with venous blood (26 of 150). An immediate explanation of this finding was not feasible but we suggest that the discrepancies between the results may be due to the inherent ability of *Plasmodium falciparum* [which is the major *Plasmodium* species in Cameroon (WHO, 2010)] infected red blood cells to cytoadhere to the endothelial cells lining blood vessels, a feature associated with malaria pathology (Miller et al., 2002; Sherman et al., 2003; Clark et al., 2004; Rasti et al., 2004). This may have an influence on the detection of malaria parasite in venous blood specimen, but finger prick to collect capillary blood has direct access to the microvasculature where you can have sequestered parasites [18]. This finding is very similar to that which has been reported by Ouedraogo *et al.* (1991). It is recommended that blood specimen be collected 6 hourly and tested to rule out diagnosis but we could not do that because participants were recruited in the Out Patient Department (OPD) of the hospital. It is therefore possible that positive diagnosis were missed especially at the time when venous blood was gotten as Plasmodia have been shown to be present in the blood in great numbers at certain periods (Ferri, 2009). This therefore implies that chances to have a positive diagnosis irrespective with the time are higher with capillary blood than venous blood.

From the study, we observed that the prevalence of malaria parasite drops from 29.3% to 17.3%, a difference of 12% which is huge. This has a direct implication to epidemiologic studies which employ the use of light microscopy for diagnosing malaria including the annual statistics presented by the WHO. It is therefore necessary that all studies reporting figures on malaria prevalence determined by microscopy should state the source of specimen whether capillary or venous blood smears.

In order to determine the impact of capillary or venous blood on the parasitaemia, parasitaemia was determined using standard protocol (Cheesbrough, 2006). We observed that the mean parasitaemia with capillary blood (3935 parasite/mm^3) was higher than that of venous blood (3407 parasite/mm^3). However, the difference was not observed to be significant statistically (P=0.8999). This is contrary to what Ouedraogo *et al.* (1991) observed. No significant correlation was observed between capillary and venous blood parasitaemia (r = 0.3497, P = 0.1842). Therefore it may not be easy to determine the threshold of venous blood parasitaemia at which malaria parasites are present in the capillary blood and vice-versa. This may largely depend on the intrinsic characteristics of the parasite.

5. Conclusion

Our findings suggest that usage of capillary blood to detect malaria parasites is more sensitive than venous blood and may not be readily influenced by the time of diagnosis and certain inherent properties of the parasite. These findings could have an immediate implication which may impact routine clinical practice hence improve on the management of malaria in endemic areas. From this study, it is therefore evident that the rate of positive cases drops from capillary to venous blood. It is therefore necessary that studies which employ light microscopy for detection of malaria parasites should state whether blood smears are prepare from capillary or venous blood.

References

Cheesbrough, M., 2006. District laboratory practice in tropical countries. Part 2. Second edition. Cambridge University Press. PP 44-45.

Clark, I.A., Alleva, L.M., Mills, A.C., Cowden, W.B., 2004. Pathogenesis of malaria and clinically similar conditions. Clin Microbiol Rev. 17, 509–39.

Coleman, R.E., Sattabongkot, J., Promstaporm, S., Maneechai, N., Tippayachai, B., Kengluecha, A., Rachapaew, N., Zollner, G., Miller, R.S., Vaughan, J.A., Thimasarn, K., Khuntirat, B., 2006. Comparison of PCR and microscopy for the detection of asymptomatic malaria in a Plasmodium falciparum/vivax endemic area in Thailand. Malaria Journal. 5, 121-127.

Collins, W.E., 2012. *Plasmodium knowlesi*: A malaria parasite of monkeys and humans. Annual Review of Entomology. 57, 107–21.

Ferri, F.F., 2009. Chapter 332. Protozoal infections. *Ferri's Color Atlas and Text of Clinical Medicine*. Elsevier Health Sciences. p1159.

Kilian, A.H., Metzger, W.G., Mutschelknauss, E.J., Kabagambe, G., Langi, P., Korte, R., von Sonnenburg, F., 2000. Reliability of malaria microscopy in epidemiological studies: results of quality control. Tropical Medicine and International Health. 5(1), 3-8.

McKenzie, F.E., Sirichaisinthop, J., Miller, R.S., Gasser, R.A. Jr., 2003. Wongsrichanalai C: Dependence of malaria detection and species diagnosis by microscopy on parasite density. The American Journal of Tropical Medicine and Hygiene. 69(4), 372-6.

Mendis, K., Sina, B., Marchesini, P., Carter, R., 2001. The neglected burden of Plasmodium vivax malaria. The American Journal of Tropical Medicine and Hygiene. 64 (1-2 Suppl), 97-106.

Miller. L.H., Baruch, D.I., Marsh, K., Doumbo, O.K., 2002. The pathogenic basis of malaria. Nature. 415, 673–679.

Milne, L.M., Kyi, M.S., Chiodini, P.L., Warhurst, D.C., 1994. Accuracy of routine laboratory diagnosis of malaria in the United Kingdom. Journal of Clinical Pathology. 47(8), 740-2.

Moody, A., 2002. Rapid diagnostic tests for malaria parasites. Clin Microbiol Rev. 15, 66.

Nayyar, G.M.L., Breman, J.G., Newton, P.N., Herrington, J., 2012. Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa. Lancet Infectious Diseases. 12 (6), 488–96.

Noedl, H., Yingyuen, K., Laoboonchai, A., Fukuda, M., Sirichaisinthop, J., Miller, R.S., 2006. Sensitivity and specificity of an antigen detection ELISA for Malaria diagnosis. The American Journal of Tropical Medicine and Hygiene. 75(6), 1205–1208.

Ouedraogo, J.B., Guiguemde, T.R., Gbary, A.R., 1991. Etude comparative de la densite parasitairede Plasmodium falciparum dans le sang capillaire et dans le sang veineux chez des porteurs asymptomatiques (Région de Bobo-Dioulasso, Burkina Faso). Médecine D'afrique Noire. 38 (8/9).

Rasti, N., Wahlgren, M., Chen, Q., 2004. Molecular aspects of malaria pathogenesis. FEMS Immunol Med Microbiol. 41, 9–26.

Sarkar, P.K., Ahluwalia, G., Vijayan, V.K., Talwar, A., 2009. Critical care aspects of malaria. Journal of Intensive Care Medicine. 25 (2), 93–103.

Sherman, I.W., Eda, S., Winograd, E., 2003. Cytoadherence and sequestration in Plasmodium falciparum: defining the ties that bind. Microbes Infect . 5(10), 897-909.

Snow, R.W., Guerra, C.A., Noor, A.M., Myint, H.Y., Hay, S.I., 2005. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature. 434 (7030), 214-217.

Warhurst, D.C., Williams, J.E., 1996. ACP Broadsheet no 148. July 1996. Laboratory diagnosis of malaria. Journal of Clinical Pathology. 49(7), 533-8.

WHO, 2010. Cameroon: Epidemiological Profile. World Malaria Report. WHO, Geneva, Switzerland.

Wongsrichanalai, C., Barcus, M.J., Muth, S., Sutamihardja, A., Wernsdorfer, W.H., 2007. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). The American Journal of Tropical Medicine and Hygiene. 77(6), 119-27.

World Meteorological Organisation (WMO), 2012. Climatological information. Cameroon. Douala. [http://worldweather.wmo.int/055/c01513.htm].