IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 3 Ver. IV (Mar. 2015), PP 68-71
www.iosrjournals.org

Modified Method for Dehemoglobinization of Thick Smear for Malaria Diagnosis

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Abstract: *When malaria is suspected in a patient, a definitive result indicating the presence or absence of parasite, preferably showing the species should be available within 1-2 hours of receipt of the blood specimen in the laboratory. This is especially important if falciparum malaria is suspected. By the conventional method it is impossible to give a report within this short period if the parasite density is low and difficult to identify in thin smear. Thick smear preparation and staining takes more than two hours. The species diagnosis may not be possible from thick smear due to distortion of morphology. In all such cases subsequently thin smear has to be screened for a long time to detect a parasite and identify its species.*

In this paper two different methods for rapid thick smear preparation and staining are described which will avoid the necessity of thin smear examination and thus shortening the turnaround time. In the first method acetic acid is used to lyse the RBCs in an isotonic solution to prevent the osmotic effect of water on the cells and parasites. In the second method acetic acid is used on fixed cells which resist distortion.

Keywords *- Malaria, dehemoglobinization, thick smear, rapid diagnosis, diagnosis of malaria.*

I. Introduction

Malaria is one of the oldest diseases in the human history. Disease with symptoms similar to malaria was present around 2500 BC [1]. Hippocrates has described a disease with symptoms of malaria. In India Sushrutha who lived around 600 BC has mentioned of a disease with symptoms similar to malaria. Malaria is reported from every continent other than Antarctica [2, 3].

In spite of the advances in the diagnosis and treatment of malaria it is still prevalent in many parts of world. There is high incidence of malaria in some parts of India and sporadic cases occur throughout the country. In states like Kerala the incidence was very low, but due to migration of laborers from other states, many cases occur including some indigenous cases. Number of cases with mixed infection is also seen increasingly.

WHO advocates making parasite based diagnosis of malaria in all suspected cases before starting the treatment. In areas where facility for good microscopy is not available, rapid diagnostic tests for malaria are of great use. The disadvantages of such tests are that all kits may not be standardized. However some studies [4] have claimed that when high quality rapid diagnostic tests (RDT) are used, the performance is better than microscopy under standard conditions. Another study [5] showed that RDTs are comparable to PCR in accuracy with the advantage of rapidity. They also argue that it was superior to microscopy by experts. But the fact remains that the RDTs will detect only Plasmodium vivax and falciparum; other species may be missed in areas where they are present. If the parasite count is low the RDTs may give false negative result. This warrants that all negative cases should be examined by microscopy before giving a definite negative report.

Other tests like PCR and nucleic acid sequence based amplification (NASBA) are useful and more sensitive than thick smear [6], PCR gives a positive test even when the parasite density is 5/µl [7, 8]. RDT and thin film give positive test only when parasite density is more than 100-200/µl while thick film gives positive test when density is 50/µl. The major disadvantage of PCR and NASBA is that they are not readily available in most of the areas and are not affordable to the class of people suffering from the disease.

These facts show that in spite of all developments in the field of malaria diagnosis, demonstration of parasite in thick or thin smear remains as the gold standard for diagnosis.

When thin smear is examined, at least 200 to 300 oil immersion [9, 10] fields has to be examined to declare it as negative, it has been found that consumption of prophylactic drugs can cause a reduction in number of parasites[11]. In such cases more fields should be examined before giving a negative result. Falciparum malaria is a potentially lethal condition and in some cases the parasites are largely confined to internal organs and only very scanty organisms is seen in peripheral blood. In such cases much time should be spent in ruling out infection. If one smear is negative more smears should be examined during a period of 36 hours.

Thick smears contain about 20 times more blood than thin smear and so proportionately more sensitive in detecting the parasites when compared with thin smears.

The main disadvantage of thick smear is that during dehemoglobinization much distortion can occur to the morphology of parasite. The slow drying of smear can also induce change in morphology of parasite. It may

be difficult to identify the species from thick smear alone. So a thin smear has to be subsequently examined to identify the species. Another drawback of thick smear is that after preparation of smear it may take at least one to two hours for through drying, another 15 minutes for dehemoglobinization and one more hour for giemsa staining. Sometimes the smear may peel off on staining and the entire procedure may have to be repeated. This will increase the time needed to give the final report. This may be critical if the patient is suspected to be having CNS malaria.

This paper proposes two modified methods of dehemoglobinization and staining of thick smear. These methods will reduce the preparation time and also preserve the morphology of parasite. So detection and species diagnosis can be given from the thick smear alone avoiding need for a thin smear examination.

II. Method

Two different methods were presented in this paper for removal of hemoglobin with minimum distortion of the morphology.

2.1 Method-1

The thick smear was prepared by the conventional method. One large drop of blood was spread using the corner of another slide to get a 2 cm square smear. The smear was allowed to dry completely for about 30 to 60 minutes.

When drying was complete, dip the smear in a solution prepared by adding 10 drops of glacial acetic acid to 50 ml of normal saline in a Coplin jar for a few seconds. Repeat the procedure a few times till dehemoglobinization is complete. Alternatively the smear can be placed on a horizontal surface and cover the surface with the same acetic acid in normal saline solution. Wait for a few seconds, pour off the solution. If dehemoglobinization is not complete, repeat the procedure till the slide is colorless. Drain it completely and wash with normal saline for a few times to remove traces of acid. Only small quantity of solution is needed for this method and the solution is stable for a long time at room temperature.

Pour dilute giemsa or leishman stain according to the preference and stain for required time as for blood smears.

2.2 Method-2

Prepare thick smear as usual. Without drying, immediately put it in 95% ethyl alcohol or 85% isopropyl alcohol for 15 minutes. When the fixation is complete take it out and keep on a horizontal surface. Pour a few drops of glacial acetic acid. Wait for a few seconds. When dehemoglobinization is complete wash it thoroughly with water till all traces of acid is removed. Preferably it can be dipped in lithium carbonate solution followed by washing in water. Stain the slides by rapid dips by Papanicolaou method. Dehydrate and mount in DPX.

III. Result And Discussion

By both methods, most of the areas of smear were completely dehemoglobinized and stained well as seen in Fig.1, Fig.2 and Fig.3. Only very thick areas showed some residual hemoglobin. By prolonging the time of dehemoglobinization this also could have been removed. The morphology of all forms of malarial parasites were well preserved so also the morphology of white blood cell nuclei. The malarial pigment was also seen clearly. No osmotic distortion of parasite morphology was seen in the smears unlike in the hypotonic solution method. Crisp outlines of RBC membrane were seen and the RBCs were transparent. The preservation of morphology of parasite made it easy for identification of species. Multiple rings of P. falciparum also could be easily identified.

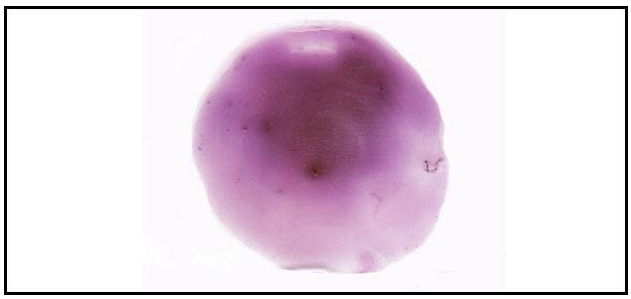

Fig.1 Wet fixed, PAP stained thick smear

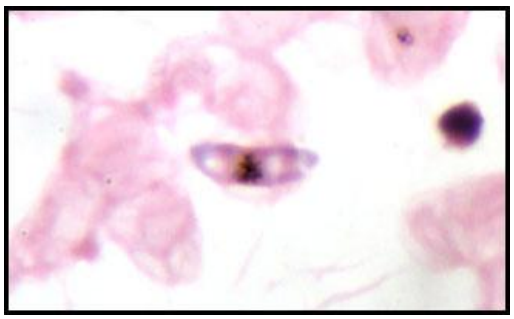

Fig.2 Gamatocyte of P. falciparum (Oil Immersion) in a background of transparent RBCs

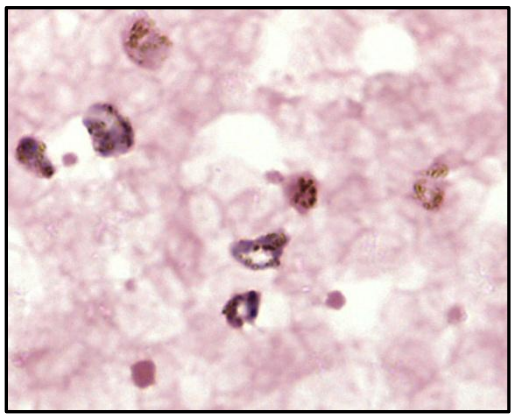

Fig.3 Trophozoites of Plasmodium vivax with pigment

The common artifact seen in thick smear is swelling of parasite and distortion of morphology making it difficult to identify the correct species even for experts. This makes it mandatory to examine thin smears for the correct diagnosis wasting precious time [12, 13]. This distortion of morphology is seen in the nuclei of white blood cells also. When the first method was used, the morphology of malarial parasites and the white cell nuclei were better preserved with sharp margins than the conventional method. No distortion due to the hypotonic swelling effect was seen. This is the main advantage claimed for this method. There was no change in the time required for completion of the preparation and staining of thick smears. But in most cases the species diagnosis could be made from the thick smear itself. There was no need to waste time in examining the thin smear. The total turnaround time for issuing the final report could be reduced. When the second method was used the smears were wet fixed immediately. The time needed for drying of the smear could be eliminated. The alteration in morphology of parasite induced by slow drying of thick smear also could be avoided. Since the acid treatment is done after fixation of cells no further alterations in shape occurred. Some cases of mixed infection missed by routine examination of smear could also be picked up by the modified method. In the conventional thick smear preparation and staining method there is no fixation of cells during any of the steps. Hence the quality of smear will deteriorate quickly and it cannot be used for future reference. In the present method described, the cells are well fixed and so they are resistant to deterioration. They can be stored for longer lime and can be used for future reference.

IV. Conclusion

This paper proposes a modified method for dehemoglobinization of thick smear for rapid diagnosis of malaria with the following advantages

1. In the modified method described here the morphology of parasites could be better appreciated in thick smear making it unnecessary to examine thin smear.
2. When thick smears are wet fixed, much time could be saved by eliminating the time needed for drying of thick smear.
3. Due to fixing of smear, the storage life of smear is increased, making it available for future reference.

In emergency situations it is possible to issue the report in less than one hour. It is a great advantage when the parasite density is very low as in some cases of falciparum malaria or when the suspected patient has taken prophylactic treatment.

References

[1]. Neghina R, Neghina AM, Marincu I, Iacobiciu I (2010). "Malaria, a Journey in Time: In Search of the Lost Myths and Forgotten Stories". Am J Med Sci**340** (6): 492–498. doi:10.1097/MAJ.0b013e3181e7fe6c. PMID 20601857

[2]. Carter R, Mendis KN (2002). "Evolutionary and historical aspects of the burden of malaria". ClinMicrobiol Rev**15** (4): 564–94. doi:10.1128/cmr.15.4.564-594.2002. PMC 126857. PMID 12364370.

[3]. Cox FE (2010). "History of the discovery of the malaria parasites and their vectors". Parasites & Vectors**3** (1): 5. doi:10.1186/1756-3305-3-5. PMC 2825508. PMID 20205846

[4]. de Oliveira AM, Skarbinski J, Ouma PO, Kariuki S, Barnwell JW, Otieno K, et al. Performance of malaria rapid diagnostic tests as part of routine malaria case management in Kenya. Am J Trop Med Hyg. Mar 2009;80(3):470-4

[5]. Polley SD, González IJ, Mohamed D, Daly R, Bowers K, Watson J, et al. Clinical evaluation of a loop-mediated amplification kit for diagnosis of imported malaria. J Infect Dis. Aug 2013;208(4):637-44

[6]. Mens P, Spieker N, Omar S, Heijnen M, Schallig H, Kager PA. Is molecular biology the best alternative for diagnosis of malaria to microscopy? A comparison between microscopy, antigen detection and molecular tests in rural Kenya and urban Tanzania. Trop Med Int Health. Feb 2007;12(2):238-44

[7]. Farcas GA, Zhong KJ, Mazzulli T, Kain KC. Evaluation of the RealArt Malaria LC real-time PCR assay for malaria diagnosis. J ClinMicrobiol 2004;42: 636-8.

[8]. Farcas GA, Soeller R, Zhong K, Zahirieh A, Kain KC. Real-time polymerase chain reaction assay for the rapid detection and characterization of chloroquineresistant

[9]. R1. Yu B. Examination of blood smears for malaria parasites. Toronto: Laboratories Branch, Ontario Ministry of Health and Long-term Care, 2005.

[10]. R2.Garcia LS, Johnston SP, Linscott AJ, Shimizu RY. Laboratory procedures for diagnosis of blood-borne parasitic diseases. Washington: American Society of Microbiology Press, 2008

[11]. Plasmodium Falciparum malaria in returned travelers.Clin Infect Dis 2006;42:622-7

[12]. J. Iqbal, P.R. Hiraa F. Al-Ali, N. Khalida A. Sherc. Modified Giemsa Staining for Rapid Diagnosis of Malaria Infection. Med Princ Pract 2003;12:156–159; DOI: 10.1159/000070751

[13]. Warhurst DC, Williams JE. Laboratory diagnosis of malaria. J Clin Pathol 1996; 49: 533-538