BRIEF COMMUNICATION

AUDIT ON THE LABORATORY DIAGNOSIS OF MALARIA PARASITES AT THE UNIVERSITY OF GONDAR HOSPITAL LABORATORY, NORTHWEST ETHIOPIA

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ABSTRACT

Background: *Malaria remains a heavy burden on tropical communities. In Ethiopia 65% of the population is living in 75% of the country at risk of malaria. Following correct laboratory procedures and identifying malaria parasites are of paramount importance in the laboratory diagnosis of malaria parasites. The aim of this audit was to assess the quality assurance system of malaria diagnosis in the University of Gondar (UOG) hospital against the established standards for the audit.*
Methods: *The clinical audit was conducted using a structured questionnaire, direct observation on laboratory activities, and re -examining the diagnosed slides confidentially. Standards were set for blood collection, the staining of blood films, the quality of the blood films, and reporting systems.*
Results: *Of the 214 blood samples examined for malaria parasites, 66 were true positives, 0 false positive, 7 false negatives and 141 true negative. Moreover, the wiping of the first drop of blood during capillary puncture, failure to filter Wright's staining solution on daily bases, and failure in the use of high- power objective during microscopy were among the highly violated standards with few areas of good practices.*
Conclusion: *In this audit, the false negative rate of malaria parasite microscopic examination in the UOG Hospital Laboratory which was 5% was significant. Replacing Wright's staining solution by Giemsa's stain, preparing SOPs, and establishing internal quality control programs are recommended.*

Key words*: Audit, standard, malaria, blood film, microscopy.*

INTRODUCTION

Malaria is an infectious disease caused by the parasite plasmodia. Four identified species of this parasite exist and cause different types of human malaria, namely *plasmodium falciparum, plasmodium vivax, plasmodium Ovale* and *plasmodium malariae*, all of which are transmitted by the female anopheles mosquito(1). Worldwide, there has been an estimate of 515 (range 300-660) million episodes of clinical *Plasmodium falciparum* malaria in 2002 (2).

According to WHO estimates the global incidence of malaria in 2004 was between 350 to 500 million cases (3). About 90% of the world's malaria deaths are estimated to occur in sub-Saharan African, where the majority of the infections are caused by the most dangerous species *plasmodium falciparum* (4). In Africa, a sum of over 12 billion dollars is lost every year due to malaria. The economic burden of malaria to countries, families and individuals is immense. Malaria strikes during the planting and harvesting seasons (4). However, more than one-third of the 108 malarious countries (9 African and 29 other) documented reductions in malaria cases of > 50% in 2008 compared to 2000 (5).

Over 65% of the population of Ethiopia living in 75% of the country is at risk of malaria (2, 6). There were repeated epidemics of malaria in Ethiopia. The 1958 malaria epidemic caused around 3,000,000 cases and 150,000 deaths as reported, from the northwestern part of the country (7).

Malaria undermines the health and welfare of families, endangers the survival and education of children, debilitates the active population, and impoverishes individuals and countries (8). According to MOH 2002/2003 reports, malaria is the first cause of morbidity and mortality accounting for 15.5% of Outpatient Department (OPD) visits; 20.4% of admissions and 27% of inpatient deaths. The number of people estimated to be residing in malarious areas of

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the country has shown a dramatic increase from 17.7 million in 1965 to more than 52.6 million in 2005, due to population growth and movement (9).

Gondar is one of the malaria infested areas in the country where most of the people in the district's are at risk. Out of 9322 admissions and 1584 deaths in medical wards, at the University of Gondar Hospital over a six-year period (from September 1, 1998 to August 31, 2004), 408(4.4%) of the cases and 116 (7.3%) of the deaths were due to severe and complicated malaria (4). Moreover, a high prevalence of urban malaria infection (5.3%) was reported previously from Gondar town (10).

Diagnosis of malaria by clinical symptoms alone is not reliable as it results in unnecessary expenditure and inappropriate use of anti-malaria drugs, delay in establishing correct diagnosis and treatment of patients. The role of the clinical laboratory is more pronounced in monitoring response to treatment with anti-malaria drugs, complications of severe malaria, and the emergence of drug resistance.

Malaria can be diagnosed in clinical laboratories under microscopic examination of stained blood films or using immunochromatographic tests. Due to its easy availability, simplicity of the procedures, and cost effectiveness of the staining reagents in comparison to immunodiagnostic tests, most clinical laboratories diagnose malaria by stained blood films using Giemsa's stain more frequently and Wright's stain, rarely.

Following the correct laboratory procedures and identifying malaria parasites are of paramount importance; otherwise, wrong laboratory test results will lead to the mismanagement of patients and unnecessary resource wastage, together with the loss of confidence on laboratory test results by those requesting laboratory investigations. Considering the existing problems in clinical laboratories in the country in general, and the UOG Hospital Laboratory in particular, we have conducted an audit on the laboratory diagnosis of malaria parasite at the service laboratory.

The aim of this audit was to assess the quality assurance system of malaria diagnosis in the Clinical Laboratory of UOG Hospital against the established standards which include the sample collection methods, blood smear preparation, the staining procedure and the skill of the laboratory technician while examining blood films and identifying malaria parasite species.

METHODS

The clinical audit was conducted at the UOG Hospital Laboratory (2006/2007) using a structured questionnaire, direct observation on laboratory activities, re-examining of the diagnosed slides, and using standards without the knowledge of the laboratory technicians performing the test.

Sample size: A total of 214 blood sample handlings, preparations, and laboratory investigations of one month were audited by different instructors of the Department of Laboratory Technology organized as a team. Of 214 blood samples, 139 were collected from capillary by using blood lancets, and 75 from venous using an anticoagulant.

Standards: The following standards were considered upon conducting the audit.

Collection of blood sample: For capillary blood, rubbing the puncture site correctly and cleansing with 70% alcohol, then making a puncture of 2-3 mm deep with a sterile lancet, removing the first drop of blood, placing of a drop of blood on a clean slide, making a good smear and allowing it to air dry were considered. For venous blood, the audit standards were set after the blood sample was delivered to the laboratory. Thoroughly mixing the anti-coagulated blood within 30 minutes of collection, placement of a satisfactory drop of blood on a clean slide, and making of a good smear were used as the main standards.

Staining procedure for Wright's stain: The staining procedure audit was targeted on covering the blood smear with Wright's stain (amount of Wright's stain to be 11-12 drops), the time of staining (2 minutes initially and 3 minutes after dilution with distilled water or buffer solution), whether the buffer solution's PH was 6.4-6.5 or not, washing with distilled water until the thinner parts of the film are pinkish-red and wiping the back of the slide with dry cotton.

Microscopic examination of blood film: The audit on the microscopic examination of the stained blood films were conducted following three standards, namely observing the quality of staining reactions with 40x objective, the number of focus per each slide (about 100 focuses need to be observed before removing the slide as negative for haomoparasites), addition of a drop of immersion oil on stained blood film and examination of the preparation by 100x objectives of the microscope.

Quality of the blood film: In addition to the distribution of red blood cells during the smear preparation, the quality of the stained blood films was assessed following standards. The standards were blood films stained with Wright's stain solution. They are pinkish in color when viewed with the naked eye, and show different colors when stained in acidic and alkaline stains (Wright's stain solution is a neutral solution in its PH) which are unnecessary or bad results. On microscopic examination of the blood films, the characteristic color developed on each blood cell was also used as a standard during the audit. Accordingly, the red blood cells (pink with a central pallor), Neutrophil granules (pinkish), Eosinophil granules (red orange), Basophile granules (dark blue), Cytoplasm of monocytes (faint blue gray), Platelets (violet granules), Malaria parasites (sky blue cytoplasm and red purple chromatin dots), and laboratory diagnostic stages of malaria parasites (*plasmodium falciparum* ring and gametocyte stages and *plasmodium vivax* all stages) were used to assess the quality of the blood films.

Reporting Systems: The audit on the reporting system of the malaria parasites laboratory examination was based on the standards which include a minimum of 100 fields of microscopic observation; a minimum of 5 minutes was used before reporting the result and in the report of negative slides, as no haemoparasite was seen. Moreover, reporting the type of malaria parasite and the observed stages of the parasite in the case of *plasmodium falciparum* together with the degree of parasitemia (parasite load) for malaria parasite positive slides were also considered.

RESULTS

A total of 25 standards were used during the audit. Seventy-seven percent of the samples were collected from properly rubbed fingers, 22% from not properly rubbed, and 1% not documented (n=139). All the 139 capillaries (finger tips) were properly cleaned with 70% alcohol prior to puncture. The punctures were deep and not deep enough on 83%, and 17%, respectively, of the samples collected from capillary (n=139). Sixty-two percent of the blood samples from capillary were collected after wiping the first drop of blood, and 38% without removing the first drop of blood. Ninety-five percent of the blood samples collected with an anticoagulant were mixed properly before smearing (n=75). Fifty-six percent of the anti-coagulated blood samples were investigated within 30 minutes, 39% within 30-60 minutes, and 5% stayed for more than an hour (figure 1). The placement of a sufficient amount of blood on a slide, the use of clean or new slides, the preparation of good smears, the drying of the smear in air, and the use of smooth-edged spreader were 87%, 88%, 74% and 80%, respectively (n=214).

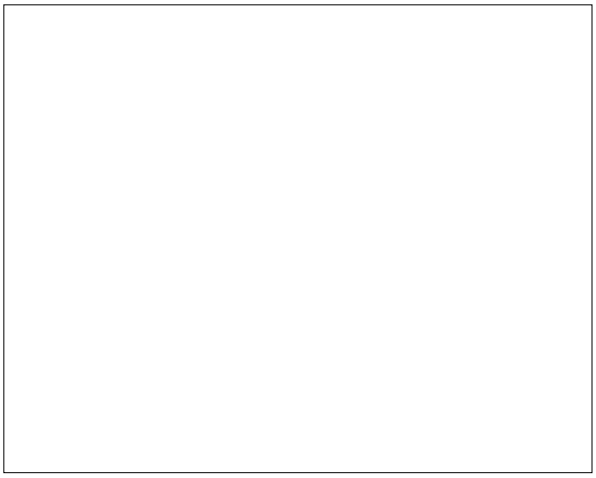

Figure 1 - *Duration of the anti-coagulated blood kept prior performing a blood smear (n=75).*

Fifty-nine percent of the blood films were stained with filtered Wright's staining solution (figure 2), and 99% of the smear was stained within 2hrs of preparing the blood smear (n=214). None of the blood samples was stained with Giemsa's stain as there were no thick blood smears prepared in the UOG hospital laboratory during this study period (2006/2007).

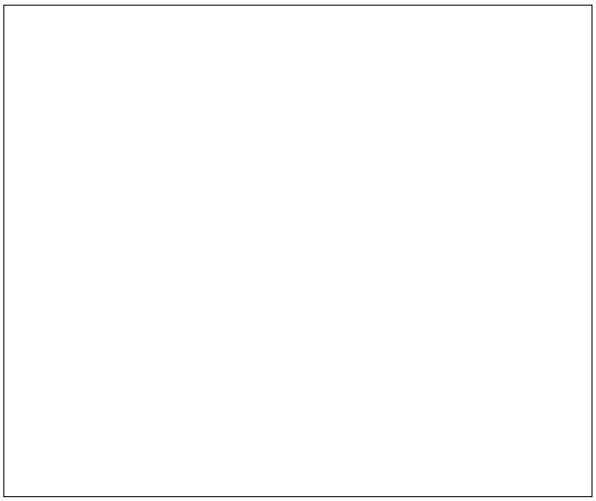

Figure 2 - *The proportion and the uses of filtered Wright's stain solution on daily bases evaluated for one month (n=214).*

Keeping Wright's stain solution on a blood smear slide before and after dilution showed a high variability among laboratory technicians in this audit (figure 3 and 4).

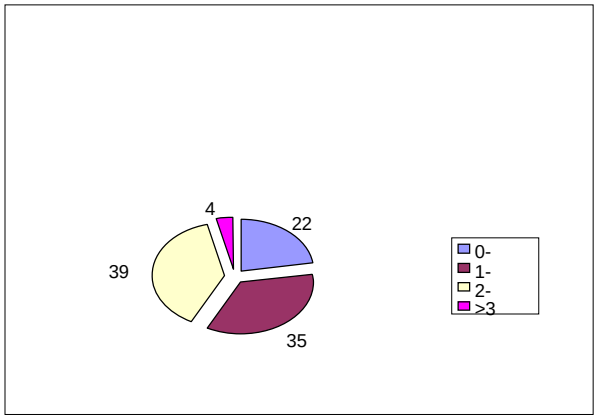

Figure 3 *- Variable duration of staining during Wright's staining procedure before diluting the stain on blood smear (n=214).*

Figure 4 *- Variable duration of staining after Wright's stain diluted with buffer/distilled water n=214).*

The practice of the laboratory technicians in washing the slides with distilled water until the thinner part of the blood film is pinkish-red, and wiping the back of the slide with dry cotton or gauze were 84% and 81%, respectively. Sixty percent of the stained smears were air-dried before examination and only 5% of the slides visualized using high power (40x) objective of the microscope. The microscopic examination time before releasing the slides negative for haemoparasite is variable among the laboratory technicians (figure 5).

All laboratory technicians reported the species of the parasite (n=68); the developmental stage of the parasite was not indicated in only 1% of the positive slides. Parasitic load is not reported in 53% of the positive cases. Re-checked slides showed that there were 66 true positives, 0 false positive, 7 false negatives, and 141 true negatives among 214 blood samples examined for haemoparasites in one month in the UOG Hospital Laboratory.

Figure 5*. The microscopic examination time of blood films among laboratory technicians prior to reporting the result as no hemoparasite or negative for hemoparasite (n=214).*

DISCUSSION

This audit has documented the profile and typical features of malaria and other hemoparasite diagnosis in the UOG Hospital Laboratory. Finger tips were not rubbed properly in 22% of blood samples collected. Massaging the finger from hand tip 5-6 times increases the blood flow. This maneuver can be best described as gently milking the finger, not squeezing it. However, care must be taken not to over do it, as it may cause erroneous results due to the concentration of tissue fluids (11). Capillary blood puncture was not deep enough on 17% of the blood samples collected during the study time (one month). According to the National Committee for Clinical Laboratory Standards (NCCLS) recommendation for puncture site for blood samples, the third or fourth (middle or ring) finger is used to obtain a blood sample. The puncture should be made perpendicular to the fingerprint ridges as the blood will more likely bead rather than rundown the channels of the fingerprints. Also the puncture devices (the depth) need to be designed to make a deep puncture (1.5mm) at the chosen site (12).

The audit showed that 38% of the capillary blood samples were collected without removing the first drop of blood. Skin puncture or capillary blood collection involves puncturing the dermis layer of the skin to access the capillary beds which run through the subcutaneous layer of the skin. Blood obtained via skin puncture is a mixture of undetermined proportions of blood from arterioles, venules, capillaries, plus interstitial and intracellular fluids (13).This dic-

tates wiping or removing the first drop of blood prior to preparing the blood smear for malaria microscopy or other hematological studies.

Thirteen percent of the blood smears were prepared by placement of insufficient amounts of blood on slides. The blood volume used should allow for a wedge blood film of an appropriate thickness of 2.5 to 4 cm in length. Typically, for the spun slides, 30µl will result in a monolayer of sufficient size, but different volumes may be required for different spinners (14). Moreover, standard blood cell investigation procedures tell us the relevance of the uses of clean microscope slide while preparing blood smears.

The hospital laboratory is currently diagnosing malaria parasites by preparing thin blood films and staining with Wright's stain solution. However, Geimsa's stain is the most acceptable and recommended stain for microscopic examinations of blood parasites. Thick and thin blood films should be prepared and examined in all cases of suspected malaria. The thick film should be used for the detection of malaria parasites and the thin film for species identification. Thin films should be fixed and stained with Giemsa's stain, and thick films should be stained unfixed after drying (14). Thick smears are 20-40 times more sensitive than thin smears for the screening of Plasmodium parasites, with a detection limit of 10-50 throphozites/µl of blood. Thin smears allow one to identify malaria species (including throphozites of mixed infections), quantify parasitemia and assess the presence of schizonts, gametocytes, and malarial pigments in neutrophiles and monocytes. The diagnostic accuracy relies on the quality of the blood smear and experience of the laboratory personnel (15).

Besides the replacement of Wright's stain for Giemsa's stain, 41% of the blood films were stained with unfiltered Wright's stain solution. Typically Wright's stain solution is prepared by dissolving 0.3 gram of Wright's stain powder in 100 ml of absolute methanol and left in a closed container at room temperature for 24 hours and must be filtered before use (13). Variable duration of staining was observed during Wright's staining in the hospital laboratory while staining blood films for malaria parasite examination (figure 4). In addition, 17% and 19% of the stained slides were not washed with distilled water until the thinner part of the blood film is pinkish-red, and the backs of the slides were not wiped with cotton or gauze, respectively. Standard Wright's staining procedure tells us to stain the blood film for 1-3 minutes prior to the addition of distilled water or phosphate buffer and to stand as long as 2-6 minutes (twice the initial staining time) after diluting the solution on the smear. It is also recommended to rinse the stained smear with distilled water or phosphate buffer until the edges show faintly pinkish-red (16, 17). The results of this audit showed that only 5% of the slides were visualized using the high power (40X) objective of the microscope. However, standard text books recommend that blood films should be examined microscopically using the 40X and 100X objectives of the microscope (18).

The microscopic examination time before releasing the slides negative for haemoparasite is variable among laboratory technicians. Published works documented that before reporting a negative result, at least 200 oil immersion visual fields at a magnification of 1000X should be examined on both thick and thin smears which have a sensitivity of (90%) (15).In small proportions (1%) of the positive slides the developmental stages of the parasites were not indicated. In falciparum malaria, parasitized erythrocytes may be sequestered in tissue capillaries, resulting in falsely low parasite count in the peripheral blood. In such instances, the developmental stages of the parasite seen on blood smears may help to assess disease severity better than parasite count alone. The presence of more mature parasite forms (> 20% of parasites as throphozites and Schizonts) and more than 5% of neutrophils counting malarial pigments indicate a more advanced disease and a worse prognosis (18). In this audit, parasitic load was not reported in 53% of the positive cases. Whenever malaria parasites are detected, the percentage of parasitized red blood cells should be quantified, cells that contain only gametocytes being excluded from the count. Quantification should be performed using a thin film and 1000 red blood cells being examined (14).

False negativity in this audit was 5 % (7/141), two false negatives on *plasmodium falciparum* positive slides with 5 false negatives on *plasmodium vivax* results. The possible reasons for more false negative results in *plasmodium vivax* microscopic examination could probably be the inability of the laboratory technicians to identify the different diagnostic stages of the parasite. This is a serious issue which highly affects the WHO strong recommendation for confirmation of a diagnosis of malaria in all suspected cases before the administration of treatments (20).

In this audit, the application of disinfectants, mixing the anti-coagulated blood before preparing blood film, and staining the blood smear within 2 hours were the areas of good practice. On the other hand, failure to wipe off the first drop of blood, to filter Wright's stain solution every day, to use buffer solu-

tion to maintain the PH of the staining solution and to use 40x (high-power) objective of the microscope were some of the highly violated standards seeking improvement. Therefore, Wright's staining techniques need to be replaced by Giemsa's stain as soon as possible, as the latter is the current golden standard for microscopic examinations of malaria parasites. Training laboratory technicians for malaria parasite microscopic examination, preparing standard operational procedures (SOPs) to avoid individual differences in the collection of blood samples, preparing blood films, staining and microscopic examination of malaria parasites, and establishing quality control programs are recommended.

ACKNOWLEDGMENT

We would like to address our gratitude to the staff of the service laboratory for their valuable cooperation which was important for this audit. We are grateful to Dr.Sisay Yifru (Line Manager of the Clinical Audit Department) and Sr. Mistir G/medhin for their valuable help in the analysis of the data of the audit.

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