الآية الكريمة

قال تعالي:

(إِنَّ اللَّهَ لَا يَسْتَحْيِي أَن يَضْرِبَ مَثَلًا مَّا بَعُوضَةً فَمَا فَوْقَهَا ۚ فَأَمَّا الَّذِينَ آمَنُوا فَيَعْلَمُونَ أَنَّهُ الْحَقُّ مِن رَّبِّهِمْ ۖ وَأَمَّا الَّذِينَ كَفَرُوا فَيَقُولُونَ مَاذَا أَرَادَ اللَّهُ بِهَٰذَا مَثَلًا ۘ يُضِلُّ بِهِ كَثِيرًا وَيَهْدِي بِهِ كَثِيرًا ۚ وَمَا يُضِلُّ بِهِ إِلَّا الْفَاسِقِينَ)

صدق الله العظيم

سورة البقرة الأية (26)

Dedication

***To the best women in the world, my mother, to my father who supports me in all my life, Greeting to my brothers and sisters, my wife and my son Mohammed Elfatih, and to all my friends*.**

ACKNOWLEDGMENTS

All praise and thanks to Allah who gave me the power to complete this work. I would like to express my gratitude and ever last appreciation to my supervisor Dr Ali Alamin and Co. Supervisor Mr. Osman Abdlgadir Osman in Kordofan University for their guidance, helpful suggestion, for solving problem, valuable supervision as well as precious advice, support continues assistance through the whole process of this research. I wish to extend my warmest thank to all members of Sudan university of science and technology (SUST) College of graduate studies specially to staff members of Parasitology and Medical Entomology department Dr.Tayseer, Mr. Galandar, Mr. Ahmed Bakheet, Mr. Amged who taught me parts of advanced Parasitology courses their continuous support and encouragement to Mohammed Osman and the other technicians staff, Iam really do not find the words that express my thanks and gratitude to him.

My sincere gratitude to Prof. Baha eldin Professor of Parasitology and medical Entomology, who taught me part of advanced Parasitology courses and continued in supporting me. Finally, I am grateful to all Laboratories participated in this study.

Abstract

External quality assessment (EQA) is an alternative tool to cross-checking of blood slides in the quality control of malaria microscopy. This study reports the findings of an EQA of malaria microscopy in El Obeid City North Kordofan.

For each medical laboratory under study, we distributed a well designed questionnaire plus five blood films (two negative and three positive films with different parasitaemia; low, moderate and high parasitaemia). We collected two slides stained & unstained blood films as well as 1 ml of Geimsa stain from each lab.

A total of 76 laboratories (55% private vs. 45% public) were participated in the study. The study found that most of laboratories are using Geimsa, but the IQC and EQA for both staff and stain demonstrated poor performance. Although only 20% have a record for malaria results. Half of laboratories reporting only whether the parasite identified or not. When studying the quality of BF; 75% are using thick blood film with acceptable quality for staining process and good for stain. The obtained results of the five blood films were; clear negative are answered correct (61%), negative with artifacts (49%), but the three positive slides were answered correctly as flows; low 49%, moderate 76% and high parasite 59%. The major errors include; not reporting the density of malaria low (50%), moderate (31%) and high parasite (13%), but those reporting wrong were low (24%), moderate (39%) and high parasite (24%). The study concluded that, the performance of medical laboratories in El Obeid in microscopical examinations for malaria parasite were acceptable, but further training courses and effective quality assurance scheme are needed.

مستخلص الدراسة

يعتبر نظام تقييم الجودة الخارجية بديلا لنظام اعادة فرز الشرائح فى عملية ضبط جودة الاختبار المجهرى للملاريا. وهذة الدراسة تبين النتائج المتحصلة من تقييم الجودة الخارجية للاختبار المجهرى للملاريا فى مدينة الابيض بولاية شمال كردفان.

تم نوزيع الاستبانات بالاضافة الى شرائح الملاريا لكل المعامل الطبية المشاركة فى الدراسة. ضمت الشرائح عدد شريحتين سلبية وثلاث شرائح إيجابية بكثافة طفيلى خفيفة ومتوسطة وعالية. كما تم اخذ اثنين مسحة دم مصبوغة وغير مصبوغة بالاضافة الى حجم 1مل من صبغة الجيمسا من كل معمل.

شاركة فى الدراسة حوالي 76 معمل (55% خاصة و 45% عامة)، اظهرت الدراسة ان معظم المعامل الطبية تستخدم صبغة الجيمسا لكنها تفتقرالى وجود نظام جودة داخلية وخارجية لتقييم كفاءة الصبغة والعاملين بالمعمل. نصف المعامل (50%) تحتوى النتيجة فيها فقط على وجود الطفيل من عدمه ، عند دراسة جودة مسحة الدم للملاريا اظهرت الدراسة أن 75% من المعامل يكتفون بالمسحة السميكة مع جودة مقبولة فى كفاءة الصبغة وعملية الصبغ. ، وحوالي (20%)منها فقط لديها دفتر لتسجيل النتائج و(80%) ليست لديهم. بينت الدراسة أن (61 %) من المعامل ميزت الشرائح السلبية بصورة صحيحة و (49%) للشرائح الساليبة المتسخة وبالنسبة للشرائح الايجابية فكانت المحصلة كما يلى: (49%)تعرفوا على الايجابية بكثافة خفيفة ،(76%) بكثافة متوسطة و59% بكثافة عالية. معظم الاخطاء كانت فى عدم تسجيل كثافة الطفيلى (50% في حالة الكثافة الخفيفة و 31% فى الكثافة المتوسطة و 13% فى الكثافة العالية)

خلصت الدراسة الى ان مستوى الاداء فى الاختبار المجهرى للملاريا فى منطقة الدراسة مقبولا، الا أن الحاجة ماسة الى مزيد من التدريب ووضع نظام ضمان جودة فعال.

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List of apperiviations

1	Ag/Ab	Antigen/ Antibody
2	DRC	Democratic Republic of Congo
3	EQA	External Quality Assurance.
4	IFAT	Indirect Fluorescent Antibody Test
5	IQC	Internal Quality Control.
6	PCR	Polymerase Chain Reaction
7	P.*falciparum*	Plasmodium *falciparum*
8	P.R	Positivity Rate
9	RDTs	Rapid Diagnostic Tests
10	WHO	World Health Organization
11	CDC	Central Disease Control

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Chapter One

Introduction
&
Literature review

Chapter one

Introduction and literature review

1.1: Introduction:

Malaria is a mosquito borne infectious disease affecting humans and other animals caused by parasitic protozoan's belonging to plasmodium (WHO, 2012a) Malaria causes symptoms that typically include fever, vomiting and headaches.(Caraballo, 2014a). The disease is most commonly transmitted by an infected female *Anopheles* mosquito, the mosquito bite introduces the parasites from the mosquito's saliva into person's blood The parasites travel to the liver where they mature and reproduce four species of *plasmodium's* can infect and be spread by humans (*P.falciparum, P.vivax, P.ovale and P.malariae*). The species *P.knowlesi* is rarely causes disease in humans.

Most deaths are caused by *P.falciparum* but the others species were caused milder form of malaria. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen based rapid diagnostic tests.

Methods that use the Polymerase Chain Reaction (PCR) to detect the parasites DNA have been developed but not widely used in areas where malaria is common due to their cost and complexity (Nadjm, et al 2012). The risk of disease can be reduced by preventing mosquito bites through the use of mosquito nets and insect repellents or with mosquito control measures such as spraying insecticides and draining standing water.

Several medications are available to prevent malaria in travelers to areas where the disease is common

1.2 Literature review :

Malaria is one of the major causes of diseases for people living in tropical and subtropical countries. It is a leading cause of morbidity and mortality in Africa (Menegon, 2010)

. The disease is caused by a haemoprotozoan parasite belongs to genus plasmodium, with mosquito of the genus Anopheles acts as a biological vector for the parasite. Malaria parasites were first discovered in the blood of a soldier suffering from malaria in 1880 by a French Army Surgeon, Charles Laveran (WHO, 2009a). In 1886 *P. vivax* and *P. malariae* were described as the causes of tertian and quartan malaria, respectively, by Camillo Golgi. Marchiafava, Bignami and Colleagues went on to describe *P.falciparum* in 1889 and were able to associate it with the most severe and lethal form of malignant tertian malaria. *P.ovale* was eventually observed in the blood of patient from East Africa in 1922.

Malaria has worldwide distribution and specially found in tropical and subtropical regions that exist in abroad band around the equator. This includes much of sub-Saharan Africa, Asia and Latin American (WHO, 2014a). Malaria is a leading cause of morbidity and mortality, in 2016 there were resulting 216 million cases and 731.000 deaths annually. Approximately 90% of both cases and deaths occurred in Africa. Malaria endemicity varies from hypo-endemic in north to hyper-endemic in the south (now the Republic of South Sudan). Sudan accounts for 50% of malaria burden in region (De Oliveira *et al*., 2009). Studies of individual hospitals in Sudan have found case fatality rates of between 12 % and 35 % with children under 5 years of age four

times more likely to die than others .

Malaria is one of the 5 diseases that mostly contribute to less than 5 years health problems in Sudan. It contributed considerably to maternal mortality which is high in Sudan at hospital level. Also it was found to be a cause of 18.1 % of low birth weight (Shillcutt *et al*., 2010).

1.2.1 Taxonomy :

Malaria parasite is classified as follows:

Kingdom: Animalia

Sub-kingdom: Protozoa

Phylum: Apicomplexa

Class: Sporozoasida

Order: Eucoccididae

Family: Plasmodiidae

Genus: Plasmodium's

Species: P. falciparum

P. vivax

P. ovaly

P. malarey

1.3 Morphology, Transmission and life cycle

1.3.1 Morphology:

Malaria parasites have sexual and asexual cycles of reproduction that completed in different host species (Cheesbrough, 2004a). While the sexual development (sporogny) occurs in the female of anopheles mosquito (definitive host), the asexual development (schizogony) occurs in man (intermediate host).

Trophozoites: The growing form of the parasite in the blood of man; it includes the ring and all stages onwards, except the fully grown gametocyte and the schizonts.

Schizonts: Form which is in process of dividing asexually; it is called "immature'' when division has just begun and "mature" when division is complete, and the parasitized cell is just rupture.

Schizogony: A process of asexually reproduction by which the nucleus and cytoplasm divide into many subsidiary parts simultaneously, each part begins called merozoites. The process occurs in the liver cells and red blood corpuscles of man.

Gametocytes: The stage of parasites containing the game. The origin of these forms is not definitely known; they are probably derived from merozoites produced by schizogony in the blood stream.

Gametes: the male gamete or microgamete, and the female gamete or macrogamete, before fertilization has take place.

Zygotes: The fertilized macrogamete.

Ookinete: A zygote capable of moving.

Oocyst: An ookinete which has settled down, become rounded and covered with a membranous cyst wall.

Sporogny: A process or cycle of sexual reproduction, which results in formation of sporozoites, this process occurs in the liver cells and the mosquito.

1.3.2 Transmission and life cycle

Development in human:

Following inoculation, the sporozoites rapidly leave the blood and enter liver cells then develop into liver schizonts then rupture to releasing merozoites which infect red cells they may be develop into ring forms then trophozoites,blood schizonts and some of trophozoites went into gametocytes which is to repeated into the new cycles (WHO, 2009b).

Early Trophozoites: It is asexual stages, known as ring stage, found in RBCs, round shaped, diameter about 3-5um, one or more chromatin dot, delicate or compact cytoplasm, stippling do not may seen in some species, diagnostic stages.

Late Trophozoites: Asexual stage, fill or nearly fill the RBCs, compact cytoplasm, differ in shape according to species, prominent stippling dot, diagnostic stage but rare in *P.falciparum*.

Schizonts: Asexual stage (Mature or Immature), fill or nearly fill the RBCs, contain merozoites, differ in number and arranging according to the species and maturity, prominent stippling dot, diagnostic stage but rare found in P.falciparum.

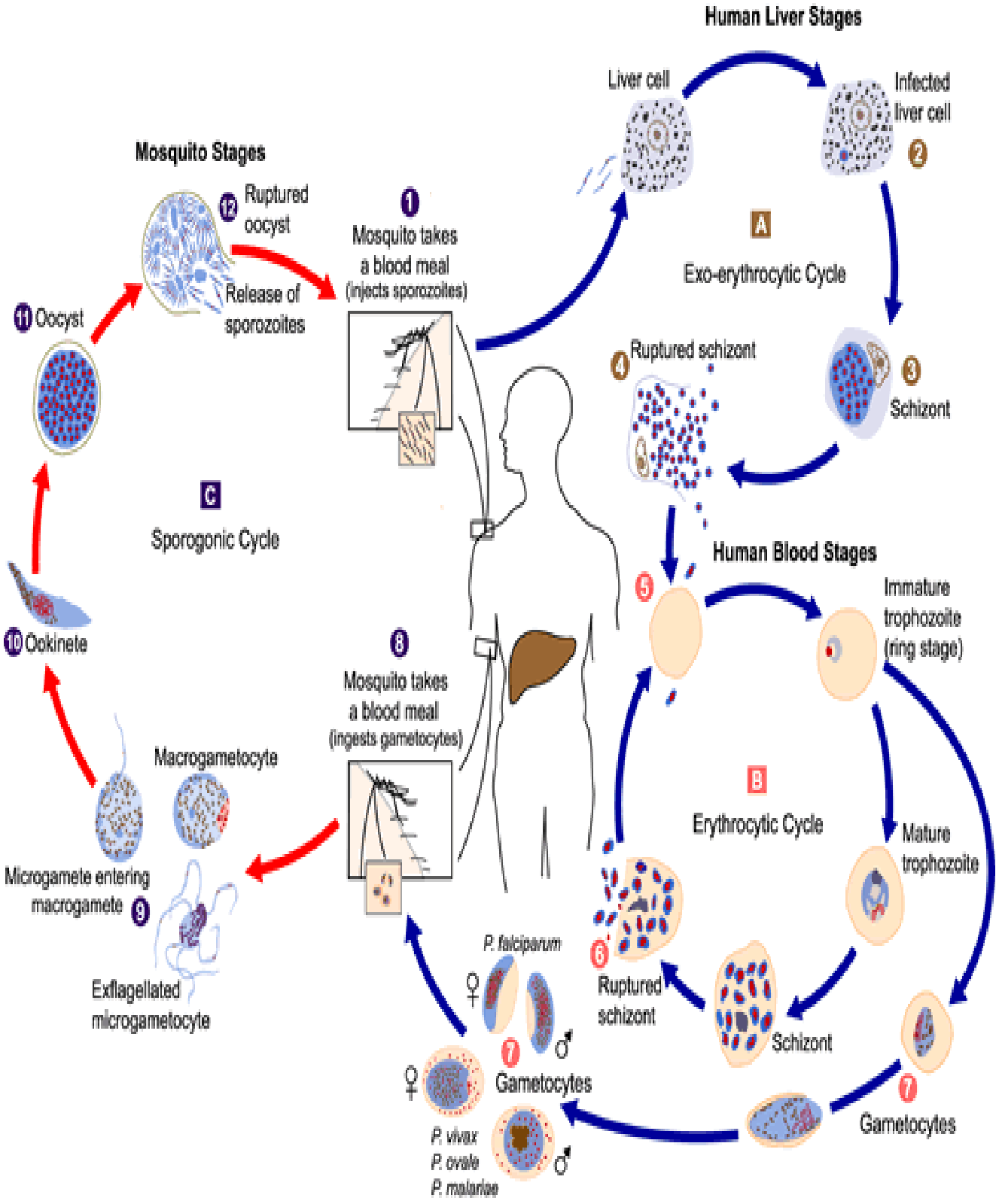
Development in mosquito:

When a female of anopheles mosquito ingest the blood of a human host containing malaria parasites all stages are digested except sexual stages (gametocytes) undergo further development in stomach of the mosquito by a projection of microgamete and macrogamete into a zygote then to ookinete to oocyst which rupture to gives sporozoites where released into the body cavity reach the salivary glands of the female of Anopheles which now becomes infective (Cheesbrough, 2004b).

The life cycle of malaria parasites is Mosquito's causes infections by bites first sporozoites enter the blood stream and migrate to the liver, they infect the liver cells, where they multiply into merozoites rupture the liver cells and return to the blood stream they which called exo-erythrocytic cycle (A) from (1-4).

The merozoites infect red blood cells where they develop into ring forms, trophozoites and schizonts which ruptured they call Erythrocytic cycle (B) from (5-7).

That in turn produced which, if taken up by mosquito develop after ingests gametocytes gives macrogametocyte and microgametocyte to ookinete, oocyst and ruptured to release of sporozoites into saprogenic cycle (C) from (8-12) then continue the life cycle again.



CDC

Figure (1): The Life cycle of Malaria

1.4 Pathophysiology

Malaria infection develops via two phases: One that involves the liver (exo-erythrocytic phase), and one that involves red blood cells, or erythrocytes (Erythrocytic phase), When an infected mosquito pierces person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the blood stream and migrate to the liver where they infect hepatocytes, multiplying asexually and asymptomatically for a period of 8-30 days. (Bledsoe, 2005). After a potential dormant period in the liver, these organisms differentiate to yield thousands of merozoites, which, following rupture of their host cells, escape into the blood and infect red blood cells to begin Erythrocytic stage of the life cycle. The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell (Vaughan *et al* ;2008) within the red cells, the parasites multiply further, again asexually, periodically breaking out of their host cells to invade fresh red blood cells. Several such amplification cycles occur. Thus, classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells. Some P.vivax sporozoites do not immediately develop into exo-erythrocytic phase merozoites, but instead, produce hypnozoites that remain dormant for period ranging from several months (7-10 months is typical) to several years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in P.vivax infections, (White, 2011) although their existence in P.ovale is uncertain. (Richter *et al* 2010). The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in

the spleen. To avoid this fate, the P.falciparum parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, there by sequestering the parasite from passage through the general circulation and spleen (Mens *et al*; 2012).

The blockage of the micro vascular causes symptoms such as in placental malaria). Sequestered red blood cells can breach the blood brain barrier and cause cerebral malaria. (Renia et al; 2012).

1.5 Clinical features and complications

Malaria causes an acute febrile illness which may be characterized by periodic febrile paroxysms which occur every 48 to 72 hours and then a patient may experience prodormal symptoms such as malaise, fatigue, headache, dizziness, joint pain, nausea, vomiting, a sensation of cold and slight fever (Cheesbrough, 2004e). Untreated malaria may lead to serious complications as in *P. falciparum* which are: cerebral malaria, anemia, hypoglycemia, pulmonary edema, black water fever, abortion and splenomegaly. Other species are less frequent or may be rare complications; *P. malariae* may are serious complications which is nephritic syndrome which may lead to renal failure, produces edema, marked proteinuria.

1.6 Diagnosis:

Malaria is a leading cause of mortality worldwide and accurate diagnostic testing for malaria can potentially save an estimated 100.000 lives annually. One of the major contributing factors to malaria mortality is delayed or in accurate diagnosis

(Iqbal *et al* ., 2012).

That is why one of the main strategic directions of the Roll Back Malaria strategic plan for Sudan, early diagnosis and treatment of malaria, is a necessary component in control of malaria (WHO, 1999a). Early diagnosis has become even more important after the emergence of drug resistance.

1.6.1 Clinical diagnosis:

This method is least expensive and widely practiced, which is based on the patients signs, symptoms and on physical findings at examination. Malaria is usually recommends prompt parasitological confirmation by microscopy or RDTs (Abba; *et al* 2011) and (Kattenberg *et al;* 2011), for all patients with suspected malaria before treatment is started. Treatment solely on the basis of clinical suspicion should be considered only when a parasitological or RDT diagnosis is not accessible. (WHO, 2009b).

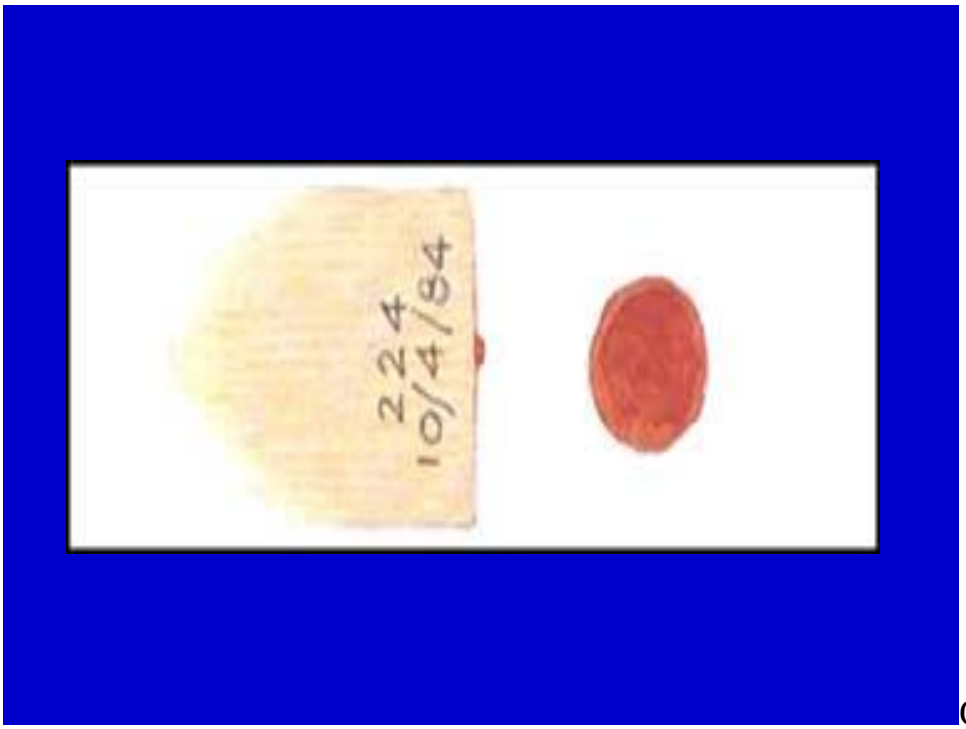
1.6.2 Laboratory diagnosis:

There are many techniques used in malaria diagnosis which are categorized into three types:

1.6.2.1 Parasitological method:

This is microscopic exam of blood smear and it is golden standard method. (WHO, 2012)

Prepare smear as soon as possible after collecting the blood from the patients (venous or peripheral) avoid any changes in parasites morphology. Then stain and micrescopical have done to see the different stages of malaria species.



CDC

Figure (2): Smear of blood film for Malaria

1.6.2.2 Serological method:

Depend to Ag/Ab reactions e.g. Indirect Fluorescent Antibody Test (IFAT) and Rapid Diagnostic Test (RDTs). (Cheesbrough, 2004c).

Examples of rapid diagnostic test used:

Histadine rich protein 2 (PfHRp2)

- Water soluble protein
- Produced by asexual stages /gametocytes, found on RBC membrane.
- Remains in PB for at least 28 days.

Plasmodial aldolase:

- Expressed by all species, can be used in combined 'P.f/P.v' tests with PfHRP2.

Parasite lactate dehydrogenase (pLDH):

- Produced by all stages of live parasites
- Present in and released from infected RBCs
- Found in all 4 human malaria species

1.6.2.3- Molecular methods:

These are based on nucleic acids detection such as PCR (Polymerase Chain Reaction) technique, and it is very high sensitive and accrued diagnosed but not widely used in areas where malaria is common as due to their complexity and expansive (Nadjm, and Behrens, 2012).

1.7 Treatment:

In tropical Africa many patients treated for mild or severe malaria do not actually have the disease, especially in adults diagnosed as having cerebral malaria, the treatment start from chloroquine drug which are the first line progressive into last Anti-malaria. (WHO, 1999b).

1.8 Evaluation of malaria micrescopical examination in Sudan

Malaria in Sudan is the major public health problem. It to an estimated 7.5- 10 million cases and 35000 deaths every year. The burden of the disease on the deaths system is a reality. Out of total outpatients, attendance, admission and deaths malaria represents 20 – 40%, 30 – 50%, and 15 – 20% respectively. These figures bring sudan on the top of WHO / EMRO countries, as Sudan shouldered 50% of cases and 70% of deaths in the region (WHO / EMRO). Malaria is endemic throughout the Sudan. The endemicity level varies from hypo endemic in the north meso endemic in the central part and hyper with holo endemic in south. *P.falciparum* was cause 85.6% of cases in 1990s. *Anopheles arabiensis* is the main vector all over the Sudan. Other efficient vectors include *A.funestus and A.gambiae*. Malaria in pregnancy (MIP) in Sudan constitutes a real problem. According to survey conducted by the National Malaria Control Programme (NMCP) in 2003.

60% of health personal working in obstetric departments admitted that malaria is public health problem. A recent published study confirmed that it was a cause of

37.2% of all material deaths at hospital level (WHO 2012b).

1.8.1-Evaluation of malaria micrescopical examination in North Kordofan:

No available data on studies carried out in this part of country. This was considered as a strong rationale to carry out the study in this area.

1.9 Prevention and Control:

Strategies to control and prevent of malaria can be through providing an accessible malaria diagnostic facilities and affordable, accessible, effective drugs to treat promptly active infections and prevent malaria in those at risk such as pregnant women and non-immune persons visiting or going to work in endemic areas (Lengeler *et al;* 2004) and (Tanser *et al*; 2010). Also, increasing public awareness of the dangers of malaria and how to reduce contact with mosquitoes. By using insecticide to kill a larval stage of mosquito, recovering all ponds and water source with oil, using a mosquito net (Raghavendra *et al*; 2011), using prophylaxis when travel to area with malaria are endemic (Palmer, 2012), breeding a special type of fish which feeding larval stage of *Anopheles*.

Figure (3a): Man spraying kerosene oil in water

Figure (3b): Indoor residual spraying

Figure (3c): Bad net

CDC

Rationale:

Light microscopy is a skills-based diagnostic procedure and accuracy depends on the microscopist competency, quality of the blood film, staining quality and the conditions of the microscope used. The reliability of field-based microscopy varies widely across and between countries, and poor standards can make diagnostic results irrelevant or even dangerous.

This study is an attempt to evaluate the current situation of malaria microscopical examination in El Obeid, North Kordofan State.

Study Objectives:

General objective:

- To evaluate the performance of medical laboratories in malaria microscopical examinations in El Obeid town, 2017.

Specific objectives:

1- To assess the efficiency of instruments and reagents used in malaria microscopical examination.

2- To assess the efficiency of those carrying out malaria microscopical examination

3- To figure out factors (if any) that influence the result of malaria microscopical examination.

4-To establish base line data for implementing quality assurance program in malaria microscopical examination in El Obeid, North Kordofan State

Chapter Two

Materials and methods

Chapter Two

Materials and methods

2.1 Study design and duration:

This is a cross sectional descriptive study that was conducted during the period March to August 2017.

2.2 Study area:

The study was carried out in El Obeid city which is located about 588 km west of the capital Khartoum.

Longitude 13.11 North and latitude 30.12 East.

It is the capital of North Kordofan state.

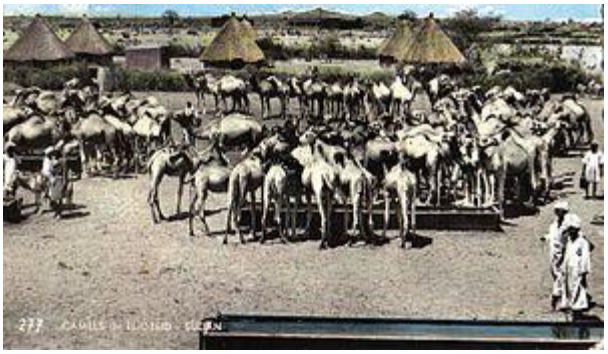


Figure (4a): El Obied

Figure (4b): Map of Sudan

2.3 Study population:

The study population included medical laboratories providing microscopical examination for malaria in El Obeid.

2.4 Sample size and sampling:

Total coverage to all medical laboratories in public and private sector that perform routine blood film for malaria. The study covered about 76 laboratories (32 public & 44 private).

2.5 Inclusion criteria:

Any laboratories that offer blood film examination for malaria in El Obeid were included in this study.

2.6 Exclusion criteria:

The criteria of exclusion based on excluding any laboratories that are not involved malaria microscopical examination.

2.7 Ethical considerations:

The health authorities at the state and locality levels were informed about the study which was only started after having their permission and. All individuals enrolled in this study were being asked to participate in the study, and an informed consent was obtained.

2.8 Data collection tools:

1- A well designed questionnaire to collect general and technical (appendix 1).

2- Result of slides that were distributed as a part of the evaluation process.

3- One stained and one unstained blood film was selected randomly as well as getting 1ml Geimsa.

2.9 Study procedure:

After having consent, the questionnaire (section A) was being completed by the interviewer. Then, a total of five slides were submitted for reading by the person who routinely performs blood film examination. These slides included; one slide with no malaria parasite, another slide with no malaria parasite, but containing stain deposits and three slides with malaria parasites; low, moderate and high parasitaemia. From each participant laboratory; one stained and unstained blood film were selected randomly as well as getting 1ml Geimsa stain. On receiving results and evaluating quality of participants unstained blood film, stained blood film and Geimsa stain, section B was then completed.

2.10 Data analysis:

All data were recorded in standard master sheets from the questionnaire that was filled by the investigator.

Chapter Three
Result

Chapter Three
Result

3. Results

The samples and Geimsa stain were examined microscopically to compare the results of each laboratory. The smears and Geimsa were examined by the well experienced and qualified investigators and the reference laboratory of malaria administration in El Obeid for further confirmation of the result. The results were as follows:

Out of 76 laborotery included in the study 44 (58%) were private lab and 32 (42%) were public lab (table 3.1).

Table No (3.1) type of lab:

Data	Frequency	Percent
Private	44	58
Public	32	42
Total	76	100

Most of laboratory personnel are holding Bsc in medical laboratory sciences, 30 (39%) , PhD or Msc 21 (28%), Diploma 17 (22%) and who had Malaria course were 8 (11%), table (3.2).

Table No (3.2) qualification of staff:

Data	Frequency	Percent
PhD or Msc	21	28
Bsc	30	39
Diploma	17	22
Malaria course	8	11
Total	76	100

The result showed the highest frequency of participants had experience more than 6-10 years 40 (53%), followed by 1-5 year 20 (26%), more than 10 years 9 (12%) and less than a year 7 (9%). table (3.3)..

Table No (3.3) Experience of micrescopist

Data	Frequency	Percent
Less than years	7	9
1-5 years	20	26
6-10 years	40	53
More than 10 years	9	12
Total	76	100

The investigation revealed that the highest result of false positive was 36 (47%) and true positive were 40 (53%), table (3.4).

Table No (3.4) the average of the positive slides per days:

Data	Frequency	Percent
True positive	40	53
False positive	36	47
Total	76	100

The study showed that the majority of participant microscopists 53 (70%) had attended the basic malaria course and 23 (30%) did not attend the course, table (3.5).

Table (3.5) Attended basic malaria course:

Data	Frequency	Percent
No	23	30
Yes	53	70
Total	76	100

The result showed that most of participant did not write full malaria report. Only 16 (21%), those who wrote present only were 38 (50%) and who wrote present + stage were 22 (29%), table (3.6).

Table: (3.6) Reporting of BFFM:

Data	Frequency	Percent
Full report	16	21
Present + stage	38	50
Only present	22	29
Total	76	100

In the table (3.7) participant who used good oil is 56 (70%) and were used bad oil is 20 (25%).

Table: (3.7) Oil immersion used:

Data	Frequency	Percent
Good	56	75
Bad	20	25
Total	76	100

The performances of different microscopes used for malaria diagnosis in medical laboratories under study were as follows:

Good is 42 (55%), acceptable 25 (34%), but about 9 (11%) were non- acceptable.

The quality of microscopes may affect the quality of malaria micrescopical examination.

Table: (3.8) General condition of the microscopes:

Data	Frequency	Percent
Good	42	55
Acceptable	25	34
Non-acceptable	9	11
Total	76	100

Our finding revealed that the majority of the medical laboratories under study didn't have internal quality control fro stain 60 (75%), but only 16 (25%) had IQC for stain, table (3.9)..

Table: (3.9) Internal quality control for stain:

Data	Frequency	Percent
No	60	79
Yes	16	21
Total	76	100

The result showed that only 14 (18%) laboratories have internal quality control and these who didn't have were 62 (82%), table (3.10).

Table: (3.10): Internal quality control for staff:

Data	Frequency	Percent
No	62	82
Yes	14	18
Total	76	100

The results showed that 34 (45%) of the laboratories have external quality control system and 42 (55%) have not external quality control, table (3.11).

Table: (3.11) External quality control for lab:

Data	Frequency	Percent
No	42	55
Yes	34	45
Total	76	100

The study showed that most of the laboratories had no duration for EQC 45 (59%), 25 (33%) had monthly duration for EQC and 6 (8%) had quarterly duration, table (3.13).

Table: (3.12) duration for EQC:

Data	Frequency	Percent
Monthly	25	33
Quarterly	6	8
No	45	59
Total	76	100

The result showed the majority of laboratories 61 (80%) don't have records for blood film, but only 16 (20%), table (3.13).

Table: (3.13) Does the lab have records:

Data	Frequency	Percent
No	61	80
Yes	15	20
Total	76	100

The result showed that most of the laboratories performed only thick blood film 60 (69%) and 16 (21%) performed both thin and thick in one slides (table 3.14).

Table (3.14): Preparation of blood film collected:

Data	Frequency	Percent
Thick	60	79
False positive	16	21
Total	76	100

Table (3.14.1), shows the quality of the blood film as 11(14%) non-acceptable, 40 (53%) acceptable and 25 (33%) as good.

Table (3.14:1): Quality of blood film collected:

Data	Frequency	Percent
Non- acceptable	11	14
Acceptable	40	53
Good	25	33
Total	76	100

Table (3.14:2) shows the result of stained blood film to assess the quality of staining techniques 18 (24%) as non-acceptable, 39 (51%) acceptable and 19 (25%) as good.

Table (3.14:2) Quality of staining:

Data	Frequency	Percent
Non- acceptable	18	24
Acceptable	39	51
Good	19	25
Total	76	100

On the other hand, quality of the Geimsa stain from the participant was 8 (11%), as non-acceptable, but 13 (17%) were acceptable and 55 (72%) are good. Table (3.15).

Table (3.15): Quality of stain:

Data	Frequency	Percent
Non- acceptable	8	11
Acceptable	13	17
Good	55	72
Total	76	100

The study showed that most of the laboratories 42 (55%) use health water for preparation, 29 (38%) use tab water and 5 (7%) were used buffered water, table (3.16).

Table (3.16): preparation of working stain:

Data	Frequency	Percent
Tab water	29	38
Buffer	5	7
Health water	42	55
Total	76	100

Five blood films were distributed for each medical laboratory to assess the performance of microscopists in microscopical examination of malaria; two films were negative (one with artifacts and another without deposits) and so as well prepared three positive films with variable parasite densities; low, moderate and high parasitaemia.

The investigation revealed that the result of negative clear who answered true is about 49 (64%) and false were 27 (36%), table (3.17).

Table: (3.17) Negative clear:

Data	Frequency	Percent
Correct	49	64
Wrong	27	36
Total	76	100

Table (3.18) shows the negative with artifacts. The result as true 39 (51%) and false 37 (49%).

Table: (3.18) Negative with artifacts:

Data	Frequency	Percent
Correct	37	49
Wrong	39	51
Total	76	100

The result for blood film with low parasites count, participant wrote true were 39 (51%) and wrong were 37 (49%), table (3.19).

Table: (3.19) Low parasites:

Data	Frequency	Percent
Correct	39	51
Wrong	37	49
Total	76	100

Table (3.20) shows the result of moderate parasites, there who wrote true result were 61 (80%) and wrong were 15 (20%).

Table: (3.20) Moderate parasites:

Data	Frequency	Percent
Correct	61	80
Wrong	15	20
Total	76	100

Table (3.21) shows the result of blood film with high parasites count . these who wrote true answer were 47 (88%) and false were 19 (12%). there who wrote true result were 61 (80%) and wrong were 15 (20%).

Table: (3.21) High parasites:

Data	Frequency	Percent
Correct	47	62
Wrong	29	38
Total	76	100

The major errors include; not reporting the density of malaria (low 52%, moderate 33% and high parasite 20%), but who reported wrong are (low 49%, moderate 20% and high parasite 12%).

Chapter Four

Discussion

Chapter four

Discussion

This study was done in El Obeid the capital of North Kordofan state and the biggest city in Kordofan region. The result will not only depend on the quality of microscopes, staining, and technique with which blood film is prepared and the parasite must be founding in the desterpioted slides but also on the concentration and motivation of technologist. This study is an attempt to evaluate the reliability of malaria microscope looking through both variation of result and associated quality assurance basics.

These basics are general condition of microscopes, qualification and experience of technologist. The study assumption is that any defect in one or more of these basics will consequently affect the reliability and accuracy of the laboratory results. The checked laboratories in this study were grouped in two public and private so the most of medical laboratories are private and may constitute almost about three quarter (personal contact). Considerable number of them were established 6-15 years ago, about half (56%) are well experienced personnel. Qualification is high (74% have an academic certificate; BSc, MSc and even PhD holders). This may be in part due to the medical laboratory school had 15 years since it was established. About two-third experienced a basic malaria course, while the majority (82%) attended refresh. Must of laboratories are used Geimsa (91%) with the correct concentration (10 % for 10 minutes & 3% for 30 minutes); while 4% use Field and 5% others stain; in spite of the severe shortage in IQC for both the stain & staff (only 14 %) In the other side the majority of them are not have EQC (55%) since the duration of EQC are monthly (33%) quarterly (8%) and (59%) are not. This could be referred to neglecting, will nestles and weak supervision. But used good oil immersion 70% with good condition of microscopes 53% which gives correct result. These can be on line with the study that done by (Abd Elmonim *et al*) in Dongola for evaluation of malaria diagnosis 2016 when using good efficient microscopes they gives low false positive 36%, and when use good quality of oil immersion also gives false positive rate low 30%.Writing a full report is of great value, but still 50% laboratories making blood film report with insufficient data.

They can agree with the study done by (Mukadi *et al* 2008) conducted in the Democratic Republic of Congo (DRC) said in the reporting of the result writing no malaria with negative and only positive for the result positive not full reported and also they used poor Geimsa stain 20% and half of them were used buffered staining solution so all of them were affected to the result and they concluded that the present EQA revealed a poor quality of malaria microscopy in DRC. The problem of such reports may result in improper prescription of antimalarial drug.

Our findings demonstrated that (80%) of laboratories don't have records for blood film results, this affect the actual estimation of malaria cases. Must of instruments are used is less efficiency (46%) and take the blood samples only thick (79%) with low quality (14%).The study has focused on the way in which blood is collected, spread, and dried and if it has any influence on sensitivity on parasite detection. The results obtained from blood films distributed to labs revealed that;75% of negative clear, negative with artifacts and low positive films were reported correctly .The ability of detection of malaria parasite was high (87%) in film with law proper. Only less than half labs were able to report parasite density correctly in films with low & moderate parasite.

Chapter Five

Conclusion and Recommendations

Chapter five

Conclusion and Recommendation

5.1 Conclusion:

From the result of the current study, it is concluded that the most frequent laboratories technicians in the area of the study were acceptable works but need more IQC as well as strong EQA

4.3 Recommendations:

1. Basic and refresh courses/ seminars/ workshops should be held

2. Regional malaria control program should design an effective supervision system and implement an updated quality assurance scheme for malaria diagnosis.

3. Motivating young graduates for more practice.

4. State Medical laboratories administration must instruct medical laboratories to participate compulsory in such surveys.

5. Further studies are needed

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Appendix

Appendix 1

Questionnaire

Sudan University of Science and Technology Collage of Graduate Studies

Medical laboratory science (Parasitology and Medical Entomology)

Serial No: _____ **Date:** ____/____/_____

Section A: General data:

(1) Type of laboratories:

(1)Private (2) Public

(2) Qualification of malaria microscopist:

(1)PhD or MSc (2) BSc (3) Diploma (4) Malaria course

(3) Experience of malaria microscopist (yers):

(1) Less than a year (2) 1-5 (3) 6-10 (4) more than 10.

(4) Attendance of basic malaria course:

(1)No (2) yes

(5) Does the lab perform I.Q.C:

(1)No (2) Yes

(6) Does the lab participate in E.Q.C for the lab:

(1)No (2) Yes

(7). Preparation of working stain used:

(1)Tab water (2) Buffer

(8). Reporting BFFM:

(1) Full report (2) presence + spp (3) only presence.

(9).The average of the positive slides per days:

(1) True positive (2) false positive

(10) General conditions of microscopes used:

(1) Good (2) Acceptable (3) Non-acceptable

(11) Oil immersions used:

(1) Good (2) Bad

(12) Does the lab have record:

(1) Yes (2) No

Section B: Technical issues

(1) Blood film is made up of:

(1) Only thick film (2) only thin film (3) both.

(2)The quality of blood film is:

(1) Non-acceptable (2) Acceptable (3) Good.

(3) Quality of Geimsa stain:

(1) Non-acceptable (2) Acceptable (3) Good.

(4)Quality of staining:

(1)Non-acceptable (2) Acceptable (3) Good.

(5) Microscpist competency:

Slide code	Result	Stage(s)	Species	Parasite density