EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 2/ May 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Evaluation of Malaria Diagnosis in Dongola City Laboratories, Northern State, Sudan

MARWA ABD EL-MONIM MERGHNI
M.Sc. student, Department of Parasitology and Medical Entomology
College of Medical Laboratory Science
Sudan University of Science and Technology, Khartoum, Sudan
TAYSEER ELAMIN MOHAMED ELFAKI[1]
Department of Parasitology and Medical Entomology
College of Medical Laboratory Science
Sudan University of Science and Technology, Khartoum, Sudan
AHMED BAKHEET ABD ALLA
Department of Parasitology and Medical Entomology
College of Medical Laboratory Science
Sudan University of Science and Technology, Khartoum, Sudan
ASHA ABBAS ELSADIG
Department of Radioisotopes
Central Veterinary Research Administration, Khartoum, Sudan
MOHAMMED BAHA ELDIN AHMED SAAD
Department of Parasitology and Medical Entomology
College of Medical Laboratory Science
Omdurman Ahlia University, Khartoum, Sudan

Abstract:

This study was conducted in the medical laboratories in Dongola city, Northern State, Sudan. The laboratories comprised the governmental, nongovernmental and private sector labs, to evaluate the result of malaria diagnosis by microscopy. 500 samples were taken by different laboratories. From each individual, a duplicate was taken for follow up by investigator. From blood sample, thick and thin smears were prepared, stained by Giemsa and examined microscopically to compare the results of each laboratory. The smears

[1] Corresponding author: talfaki@yahoo.com

were examined by the investigator; in addition some of the slides examined by the laboratories under survey were sending to the reference laboratory of malaria administration in Dongola for further confirmation of the result. The results were as follows: The rate of false positive in all laboratories reached 63%. The highest false positive results 62% were reported by the private laboratories, while the nongovernmental laboratories and governmental laboratories reported 60% and 47% false results respectively. The difference was found to be statistically significant. Although the collection of the samples was done properly, the percentage of the false positives reached 42% and when collection of the samples was done in properly, the percentage reached 43%. The study showed that when blood smears were done properly, the false positive rate reached 39% and when the blood smears were done improperly, the rate reached 46%. When the smears were improperly stained, the rate was 27% and when smears were improperly stained the rate was 66%. When the general conditions of the laboratory (building, electricity and water supply, space and cleanness) were good the false positives reached 41% in good and when the general conditions were bad, the rate reached 43%. Despite the use of good and efficient microscopes, the rate of false positives results reached 34% and21% when inefficient microscopes were used. The study showed that, when a good quality immersion oil, was used the false positives results was 36% and reached 60% when the quality of immersion oil is bad. The study showed that the false positive results reached 51% among personnel who did not receive training in malaria and 30% in those who received training in malaria. The result revealed that the false positive rate was 26% among those samples examined by university graduates, while it reached 58% among those holding diplomas. The rate reached 47% among the samples examined by mixed graduates.

Key words: Evaluation, Malaria, Dongola city Laboratories, Northern State.

INTRODUCTION:

The *Palsmodium* has a life cycle divided between a human host and insect vector, 60 species of the females of the genus

Anopheles are able to transmit malaria. The mosquitoes survive in warm, humid climate where pools of water provide perfect breeding grounds. It proliferates where awareness is low and where health care systems are inadequately developed. The global outlook for malarial infection is worsening. Currently, 40% of the world's population resides in malaria-prone areas; there are execs of one million deaths, the majority of whom are young children [(1)]. In Sudan the prevalence rate of malaria in Khartoum state was shown to be 0.1% [(2)]. Hemiedan *et al.* (2005) [(3)] conducted some investigations on Anopheles in eastern Sudan. They also aimed to investigate the morbidity pattern of malaria in the area and to establish adequate base line data for evaluation of the effectiveness of various preventive measures, including future vaccines. Epidemiology of malaria in Sudan depends upon any factors including the system of irrigation, floods and seasonal laborers for cultivations and displaced people from war affected areas. Diagnosis of malaria is still based mainly on the conventional methods i.e. microscopic diagnosis, but the question about the reliability of microscopy of malaria has been repeatedly raised by clinicians, and this is due to controversial results obtained so often from different laboratories. These false results are supposed to arise due to many factors, including usage of bad microscopes, low quality staining solutions, unsuitable immersion oils, spending insufficient time examining blood films by untrained lab-technologist. Furthermore, extension of the medical laboratory services to rural areas for increasing population coverage has been achieved in some parts of Sudan, but it is unplanned and without provision of the needed requirements for that [(4)]. Misdiagnosis may result in lives loss because severe untreated infections can be life-threatening, economic loss because of the use of expensive unnecessary drugs, development of drug resistance because of incorrect use of antimalarial drugs. This incorrect use of anti-malaria drugs may also lead to

development of chemoprophylaxis which is undesirable in areas of stable malaria transmission because is delays the development of naturally acquired immunity [5]. The main objectives of this study were to evaluate malaria diagnosis in Dongola City Laboratories, Northern State, Sudan, to evaluate the results of microscopic malaria diagnosis as regards the frequencies of the false positive and false negative results, to investigate the possible factors that influence the results in different laboratories in Dongola city such as: general condition of the laboratories, condition of the slides, stain used and staining procedure, quality of the microscope, quality of the immersion oil and qualification and training of working staff and to find out feasible solution for the problems related to misdiagnosis of malaria parasite microscopically.

MATERIALS AND METHODS:

Study design:

It is a cross-sectional study.

Study area:

The study was conducted in Dongola city, in the northern state, which is located 530 KM north of Khartoum.

Study population:

The study was carried out on 11 different medical laboratories which comprised the three governmental laboratories, three nongovernmental laboratories and five private laboratories.

Sample collection:

500 blood sample were collected and blood film were made and examined by workers at the above mentioned laboratories, follow up and confirmation of the results were made first by myself (taking blood sample and making additional thick and

thin film blood film) then blood film made was sent to the administration of laboratory in Dongola for further confirmation.

Data collection:

Questionnaire was designed to collect data to evaluate laboratory performance which included the general condition of laboratory; this included cleanness dust and flies, intensity of light and supply of water, availability of adequate space, general condition of slide as to they were clean, grease free, scratch free and clean labeling, sampling technique and quality of blood film, time of staining and changing working solution, type of stain used which included method of preparation of stock stage, amount of constituent used and steps followed to prepare the stain, place of storage of stain, time for staining and for changing work, quality of used immersion oil, quality and efficiency of microscope by examining the mechanical stage the source and intensity of light, the lenses, the condenser and iris the fine and course adjustment and qualification and training experience of working personnel.

METHODOLOGY:

Preparation of blood film:

For each patient, a new grease free and scratch free slide was used for preparation of blood film. Immediately, after collection of blood film by the technologist, the same finger prick was squeezed gently and three drops of blood for the thick blood film and a small one for the thin blood film were obtained. The thin blood film was spread immediately using a smooth edged spreader and the three drops of blood were gently mixed covering an area with a diameter of 1 cm. Using black lead pencil, the slide was labeled with patient's name and number of the thin blood film. The slide was placed to dry in horizontal

position over night, and then the thin blood film was fixed with methanol.

Preparation of Giemsa stain:

Giemsa stain stock solution:

Giemsa powder (3.8 gm) was weighted and transferred to a dry bottle of 500 ml capacity which contains about 50 glass beads. Using a clean and dry measuring cylinder, 250 ml of methanol were added to the stain, and mixed well, and then 250 ml of glycerol were added to solution, and mixed thoroughly. The bottle stain was placed in water bath in 50-60 C° for one hour then, it was stored in dark brown bottle at room temperature at dark place. For better dissolving of stain, the bottle with stain was shaken 4 times every day for 5 days. The stock solution can be kept in room temperature in well stoppered bottle for year or more.

Preparation of buffered water:

For staining blood film for malaria, it essential to have buffered water with pH=7.2 to dilute the stock solution of Giemsa stain. The buffered solution was prepared by dissolving buffer tablets in one liter of distilled water. Each buffer tablet contains 0.7 gm KH_2PO_4 and 1 gm Na_2HPO_4.

Staining of blood films for malaria:

The slides were placed back in a staining trough ensuring that all thick blood films were placed to one end upwards. Three percent working solution of Giemsa stain was prepared by adding 3 ml Giemsa stock solution to 97 ml of buffered water. The working solution was gently poured into the trough, until the slide was completely covered. The slide was left in the stain for 30 minutes; clean water was gently poured into the trough to float off debris on the surface of the stain. The remaining

stain was poured off gently and it was rinsed again in clean water and the water was poured off.

The slide was removed, and the back of each slide was wiped with clean gauze. The slide was placed in a slide rack (thick blood films down wards to drain and dry, making sure that the films do not touch the edge of drying rack).

Examination of blood films for malaria:

Stained blood films were examined using a binocular light microscope (Olympus CH20) with oil immersion lens 100x.

Examination of the thick blood film:

When the slide was completely dried, a drop of immersion oil was applied to an area of the thick film that appeared mauve colored (usually around edge). In such area, possible to see best staining of malaria parasite (chromatin stained red and cytoplasm stained blue). After focus of microscope, an area that was well stained and with the best thickness was examined for malaria parasite (the area selected should show clear or pale colored background and purple-colored neutrophil nuclei and blue colored cytoplasm). 100 microscopic fields were examined searching for malaria parasite using 100x and moving systematically from one field to another.

Examination of the thin blood films:

Thin blood films were used for identification of *Plasmodium* species. When the film was completely dry after staining, a drop of immersion oil applied the lower third of the thin films. Systematic moving from one field to another was followed so as to identify and/ or to confirm the species.

DATA ANALYSIS:

Data was analyzed using SPSS program. The Chi-squire test was used for difference in proportion. 0.05 %was taken as cut off limit for 95% statistical significance.

RESULTS:

The overall prevalence of malaria in Dongola reported by different laboratories, from the patients whom were referred by physicians was found to be 26%. After follow up and re-examination, the overall prevalence rate reported was shown to be 15% in table (1). The difference was found to be statistically significant at p=0.00. The study showed that the general condition of the labs building was suitable in 3 of inspected labs and 8 laboratories were in an unsuitable condition the percentage of false positive in suitable laboratories was 59% and in unsuitable labs was 57% in table (2). The result revealed that the number of false positive slides among examined slides constituted 62% in the private labs, while it constituted 60% and 47% in the non government and governmental labs respectively in table (3), the difference between three laboratories was found to be highly significant at p=0.00. However, the number of false negative among the examined slides constituted 88%, 85% and 95% in the governmental, non governmental and private laboratory respectively in table (4). The difference was found to be statistically significant at p=0.000. The result revealed that the collection technique was good in 9 laboratories with 42% false positive, while it was bad in 2 laboratories and false positive 43% in table (5). This difference in percentage was found to be statistically insignificant at p=0.09. The result showed that the staining technique was good in 6 laboratories and bad in 5 laboratories with false positive constitute in 27% and 66%

respectively in table (6). The difference was found to be statistically significant at p=0.00. The result demonstrated that, when using good and efficient microscope, the false positive rate was low36% while it was high 60% when using inefficient microscope in table (7). The difference was found to be statistically significant at p=0.00. Using good quality immersion oil gave false positive rate of 36% while using bad quality immersion oil resulted in high rate of false positives 60% in table (8). The difference was found to be statistically significant at p=0.00. The result demonstrated that good smearing revealed 38% false positive rate, while bad smearing resulted in 46% false positive rate in table (9). This difference was found to be statistically significant at p=0.00. The results revealed that the false positives among the university graduates constituted 26%, 58% among the diploma holders and 46% among the mixed staff (university graduates and diploma holders) in table (10). The difference was found to be statistically significant at p=0.00. The result revealed that the false positive among trained personnel in malaria diagnosis was 30% while the false positive increased to 51% among those who had no training in table (11). This difference was found statistically significant at p=0.00.

Table (1): The overall prevalence rate of malaria in Dongola

Examiner	Number examined	Number of positive slides	Prevalence
Different laboratories	500	131	26%
Investigator	500	76	15%

p=0.000

Table (2): The effect of general condition of the laboratory building on the examination result

General condition of laboratories	No. of laboratories	No. of slides	No. of positive slides	Own Result			
				True		False	
				No.	%	No.	%
Suitable	3	194	44	26	59%	18	41%
Unsuitable	8	306	87	50	57%	37	43%

Table (3): The false positives among the examined slides in different types of laboratories

Sector	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No.	%	No.	%
Governmental	3	160	32	17	53%	15	47%
Nongovernmental organization	3	140	30	12	40%	18	60%
Private	5	200	69	26	38%	43	62%
Total	11	500	131	55	42%	76	58%

p=0.000

Table (4): The false negatives among the examined slides in different types of laboratories

Sector	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No.	%	No.	%
Governmental	3	160	128	15	12 %	113	88%
Non governmental organization	3	140	110	16	15 %	94	85%
Private	5	200	131	7	5%	124	95%
Total	11	500	369	38	10 %	331	90%

p=0.000

Table (5): The effect of the collection technique on the positivity of the result

Collection technique	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No.	%	No.	%
Good	9	389	103	60	58%	43	42%
Bad	2	111	28	16	57%	12	43%

p=0.09

Table (6): The effect of the staining technique on the positivity of the result

Staining technique	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No.	%	No.	%
Good	6	322	79	58	73%	21	27%
Bad	5	178	52	18	34%	34	66%

p=0.000

Table (7): The effect of the efficiency of the microscope on the positivity of the result

Efficiency of microscope	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No	%	No	%
Good	7	328	96	62	64%	34	36%
Not good	4	172	35	14	40%	21	60%

p=0.000

Table (8): The effect of the quality of the immersion oil on the positivity of the result

Quality of immersion oil	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No	%	No	%
Good	7	328	96	62	64%	34	36%
Not good	4	172	35	14	40%	21	60%

p=0.000

Table (9): The effect of smear preparation on the positivity of the result

Smearing technique	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No	%	No	%
Good	6	317	72	44	61%	28	39%
Bad	5	183	59	32	54%	27	46%

p=0.000

Table (10): The effect of staff qualification on the positivity of the result

Staff qualification	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No	%	No	%
University graduate	4	154	42	31	74%	11	26%
Diploma	1	39	24	10	42%	14	58%
Mixed staff	6	307	65	35	54%	30	46%

p=0.000

Table (11): The effect of training in malaria diagnosis on the positivity of the result

Training	No. of laboratories	No. of slides	No. of positive slides	Own result			
				True		False	
				No.	%	No.	%
Trained	23	326	56	39	70%	17	30%
Not Trained	11	174	75	37	49%	38	51%

p=0.000

DISCUSSION:

Microscopic identification of malaria parasite is considered as the main and conventional method of malaria laboratory diagnosis, although considerable efforts have been under taken to develop new laboratory method for diagnosis of malaria parasite. The use of thick blood film examined by light microscope is the most common and reliable method [(6)]. Microscopy, however, has its own biases and limitations. Result will not only depend on the quality of the microscopy, staining, and technique with which blood film is prepared and the parasite are accounted but also on the concentration and motivation of technologist [(7)]. 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 laboratory, general condition of glass slide, qualification and experience of lab-technologist. The study assumption is that any defect in one or more of these basics will consequently affect the reliability and accuracy of laboratory results. The ideal general condition of the laboratory in which the blood film for malaria are examined is to be clean, free from dust and insect and with good intensity of light and ventilation and with adequate available space for performing an organized laboratory routine work. On evaluation of the effect of the general condition of the laboratory on the technologist performance, the result showed that with good general condition of the laboratory, the reliability of the result is not equal with the result when the checked laboratory is in bad general condition. This may be explained by the fact that, the general condition of the laboratory influences in the examination results. This result is in line with Ibrahim (2001) [(4)], who reported higher results in laboratory with good general condition. Slide used in preparing films for malaria have be clean, grease free and scratch free.

Significance was observed in result when using glass slides. It was obvious that the false positives in the private laboratories reached 62%. This might probably be due to using unsuitable slide. All slides must be clean and free from grease and scratch. Using unsuitable slide will confuse malaria diagnosis and prevent detachment and washing of thick blood film during staining process. This finding agrees with comments of WHO (1983) [8]. 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. Our result showed that 58% of blood films were truly diagnosed when the sampling technique was done in the right way. Whereas only 57% were truly diagnosed when the sampling technique was done in wrong way. This might be justified by the fact that collecting large amount of blood than that actually required may result in formation of extra thick film which cannot be easily read. In contrast, small amount of blood is not re representative for all blood constituents. Moreover, a blood film that was not sufficiently dried may be washed out during staining and washing. One of most important findings was the variation in results due to different quality of stain used. The stain was checked for their preparation, storage, and time of staining and duration of changing. The stain used is Giemsa stain. It gives the best staining of malaria parasite. The study showed that the true diagnosis was 73% when stain is good and 34% when stain was of bad quality. It is recommended that the stains should be changed frequently in a routine clinical laboratory to reduce the risk of infection and carryover of the parasite, and also to assist in the standardization of staining [9]. Using the staining solution for prolonged time without changing, may affect the purity of stain by continuous dipping of the films and removal of parts of these films inside the solution. These exposed solutions may grow fungi and ciliates. Dust and dirt may also find their way to staining jars. An efficient microscope

is characterized by complete controlled mechanical stage, built in source of illumination with a good intensity of light, perfectly function fine and coarse adjustment knobs, good light directing condenser and iris, scratch free and oil cleaned lenses and the microscope should be well protected from dust and fungal growth. The microscopes of checked laboratories were examined for their efficiency. Result showed significance between efficient and inefficient microscope. Our result revealed that the more accurate result were detected when the general condition of the microscope was good and false result were likely to be due to the bad general condition of the microscopes used in this laboratory. The immersion oil used in microscope in order to avoid the bending effect of air on the beam of the light and its limitation on the objective, as mentioned by Cheesbrourph (1998) [5]. Whenever possible, the immersion oil recommended by manufacturer of microscope should be used but actually what happens is that some lab-technologist use different types of fluids to the immersion oil in order to increase its amount or using alternatives which change the oil refractive index and by turn results in scattering of the light beam and losing the details of the blood films. The study revealed that malaria parasites are easily detected and identified by trained lab-technologist. Central laboratory staff should be encouraged, to train and supervise local community health workers in the laboratory techniques required to confirm a diagnosis of malaria. This will help to ensure that malaria is diagnosed correctly and at an early stage [5]. The effect of training on microscopic diagnosing of malaria was evaluated. Considerable variation in the result was observed when blood films were examined by either trained or untrained lab-technologist. The study showed that qualified and well trained lab-technologist has got a better performance than untrained lab-technologist. This in fact is due to experience gained during in-service training. The diagnosis of malaria is still sometimes difficult

because of the sensitivity of microscope screening at low level of parasitaemia. In this study, it was clearly observed that the false negative results are more frequently recorded when the parasite count is low. The checked laboratories in this study were grouped in to three groups governmental, non governmental and private laboratory. Evaluation of their performance was in accordance with their type. It has been found that governmental laboratory have recorded the best result 53% of true positive result, this result was in agreement with Ibrahim (2001) [(4)].

CONCLUSION:

This study concluded that accurate malaria diagnosis requires well trained lab-technologist, good stain, good microscope and high quality immersion oil. The high percentage of false positive results will lead to unnecessary treatment. False result will influence adversely the implementation of an efficient control program.

REFERENCES:

1. World Health Organization (WHO). Second Inter Country Meeting of National Malaria Programme Manager. Muscat, Oman. March 2002; 24-28.
2. Salim, E. A. and Harbi, S. O. Malaria administration parasitic investigation for malaria. 2005.
3. Himeidan, Y., Elbashir, M. I., El-Rayah, E. A. and Adam, I. Epidemiology of malaria in New Halfa, An irrigated area in eastern Sudan. *Eastern Mediterranean Health Journal*. 2005; **11**(3):25-30.

4. Ibrahim, R. Causes of false result in microscopic diagnosis of malaria in Khartoum state. M.Sc. thesis. Faculty of Pharmacy. University of Khartoum. 2001.

5. Cheesbrough, M. District laboratory practices in tropical countries. 1st edition. Cambridge Shire. UK. 1998; 246-251.

6. Kilian, A. H. D., Metzger, W. G., Mutschelknauss, E. J., Kabagambe, G., Langi,P., Krote, and Sonnenhburg, F. Reliability of malaria microscopy in epidemiology studies. *Tropical medicine and international health*. 2000; **5**(1):3-8.

7. Payne, D. Used and limitations of light microscopy for diagnosis of malaria at the primary health care level. *Bulletin of the world health organization*. 1988; **66**:621-626.

8. World Health Organization (WHO). Bench aids to human malaria diagnosis. 1983.

9. Shute, G. T. (1988). The microscopic diagnosis of malaria. *Malaria principle and practice of essential malariology*. 1988; **1**:782- 813.