EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 10/ January 2016

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

ISSN 2286-4822
www.euacademic.org

Evaluation of Malaria Diagnostic Methods in Medical Military Hospital, Khartoum State, Sudan

SUHA ELHAJ SULIMAN HAMZA
M.Sc. student in 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

Abstract:

This study aimed to evaluate malaria diagnostic methods in Medical Military Hospital-Khartoum State. A Cross-sectional study design was conducted in the period between March- December 2015. The study was conducted on 200 subjects, 92 (46%) were males and 108 (54%) were females. The age ranging between 1-65 years old, the mean age was 27±18 years old. Two hundred blood samples were taken from all study subjects; clinical and parasitological data were obtained and recorded. Out of 200 blood samples, 20 (10%), 25 (12.5%) and 20 (10%) were positive for P.falciparum by using stained blood films, immunochromatographic test (ICT) and buffy coat concentration technique respectively. And out of 200 blood samples, 4 (2%) were positive for P. vivax by using the three methods. The study demonstrated that the prevalence rate of malaria in males was 13

[1] *Corresponding author: talfaki@yahoo.com

(6.5%) which was higher than in females 11(5.5%).The study showed that the prevalence was higher (12 (6%)) in age group between 1-15 years. The prevalence rate of malaria was 24(12%) by using blood films and buffy coat concentration technique, while 29(14.5%) by using ICT. This study concludes that very high sensitivity (100%) for the blood films and buffy coat concentration technique compared to ICT (96%) for malaria diagnosis.

Key words: Malaria, Immunochromatographic test, Buffy coat concentration technique, Sensitivity.

INTRODUCTION:

Malaria is a disease of global importance that result in 300-600 million cases annually and an estimated 2.2 billion people are at risk of infection [(1)]. Numerically the most important of the life threatening protozoan disease is malaria, which is responsible for at least 750,000 deaths a year, mostly in young children in Africa [(2)]. Over half of the world's population is at risk from catching malaria. Malaria is currently endemic in 109 countries in four continents and of the 500 million cases of malaria estimated to occur annually, approximately one million results in death. Most of the fatalities are in children under the age of five years old and pregnant women [(3)]. Malaria accounts for at least $12 billion in economic losses each year in Africa and a reduction in annual economic growth estimated at 1.3 percent [(4)]. The pathogenic effect of a malarial infection have been considered to be directly related to hemolysis of infected red cell and uninfected cell, liberation of the metabolites of the parasite and the immunologic response of the host to this antigenic material and the formation of malarial pigment, additionally, in *falciparum* malaria the phenomenon of cytoadherence is basic to the locally diminished tissue perfusion seen in it is more severe complications, cytoadherence is the

result of the expression on the surface of the parasitized red cell of strain and stage-specific parasite-derived ligands, which adhere to a specific receptor complex on the endothelial cells. In persons subjected to repeat attack of malaria anaemia is disproportional to the number of red blood cells infected, and indicates that non infected red blood cells may become sensitized and be destroyed [(5)]. There is a high burden of malaria-related morbidity and mortality in Sudan. However, the national malaria control programme, with WHO's support, has reduced the number of malaria cases from more than four million in 2000 to less than one million in 2010. Between 2001 and 2010, the number of deaths due to malaria reduced by 75%. WHO works in close collaboration with the national malaria control programme to implement appropriate and cost-effective malaria control interventions. These include the distribution of artemisinin-based combination therapy treatments, rapid diagnostic tests and long-lasting insecticidal nets, and the introduction of the home-based management of malaria strategy. Artemisinin-based combination therapy treatments: in 2011, around 4666 health facilities provided free artemisinin-based combination treatments. This was 89% of the total number of health facilities targeted. First introduced in Sudan in 2005, artemisinin-based combination treatments are recommended as the first-line treatment for malaria caused by *P.falciparum*, the most deadly of parasites that infect humans. Rapid diagnostic tests: to help detect malaria parasites in human blood promptly, rapid diagnostic tests were distributed to health facilities in villages. The number of health facilities with rapid diagnostic tests has reached 3363 or 73% of the total targeted facilities. Long-lasting insecticidal nets: considered as the most effective intervention, WHO has been supporting the free distribution of long-lasting insecticidal nets to families in risk areas. Home-based management of malaria: In Sudan's far-flung villages, access to curative and diagnostic services is

limited. The home-based management of malaria has been identified as one of the strategies to reduce the burden of malaria, especially in malaria-endemic areas. So far the strategy has been introduced in 988 villages across the country. With home-based management of malaria, diagnosis and treatment has been brought nearer the home and the community, so that treatment can be given within 24-hours of the onset of symptoms [6]. The main objectives of this study were to evaluate malaria diagnostic methods in Medical Military Hospital-Khartoum State, to detect the prevalence of malaria using blood films, ICT and buffy coat technique in the study area, to evaluate the efficiency of blood films, rapid diagnostic tests and buffy coat technique in the detection of malaria parasite and to compare between malaria prevalence and sex, age and symptoms.

MATERIALS AND METHODS:

Study design:

Cross-sectional study design.

Study area and study period:

The study was conducted at Medical Military Hospital-Khartoum State, in the period between March- December 2015. Khartoum State lies between longitude 31.5-34 east and latitude 15-16 north in an area about 28.165 square kilometers. It is bordered on the north and the east sides by the River Nile State, on the north-western side by the Northern State and on the eastern and southern sides by Kassala, Gedaref and Gezira States [7].

Study population:

The study was carried out on patients that clinically suspected to have malaria.

Sample size:

The sample size was obtained according to the following equation:

$N = t^2*P (1-p)/M^2)$

N = Sample size

t = the normal standard deviate (t = 1.96)

P = the frequency of occurrence of malaria (16%)

M = degree of precision (0.05%)

N = 1.96x1.96x.23x(1-.16)/.05x.05 =206

According to the above finding, the study was conducted on 200 clinically suspected patients.

Sampling:

Two hundred blood samples were collected from all participants. The blood specimens obtained by venipuncture and collected in (EDTA) anticoagulant-coated tubes. And 200 questionnaires were filled by participants. Each specimen had been tested by the three methods (blood films, ICT and buffy coat concentration technique).

Data collection:

Designed questionnaire contained the following variables: Gender, age, symptoms, previous infection, duration of previous infection, previous treatment, duration of previous treatment and parasitological results.

METHODS:

Stained blood films:

Thick blood films:

Three drops of blood were added to clean and dry slide, mixed and allowed to dry. Then the slides were stained by 10% Giemsa stain, washed and air dried. Then a drop of oil was added and examined under microscope. The number of

parasites were counted and reported by using the following grading as described by Cheesbrough [8]:

1-10 per 100 high power fields ……. +
11-100 per 100 high power fields ……. ++
1-10 in every high power field ……..+++
More than 10 in every high power field …++++

Thin blood films:

A drop of blood was added below the level of slide (2/3) and by spreader the blood was pushed forward with suitable speed, allowed to air dry, then fixed with absolute methanol, allowed to air dry and stained with 10% Giemsa stain. Then washed and allowed to dry, drop of oil was added and examined under microscope (100x oil immersion).

Immunochromatography test (ICT):

Five µl of whole blood was added into sample well in the pf/pv antigen test kits, two drops (80µls) of assay buffer were added into the developer well. Then the results were read in 20 minutes as follow: the presence of two color bands ″c″ and ″pf″, indicates a positive result for *P.falciparum*, two color bands ″c″ and ″pv″ indicates a positive result of *P.vivax*, three color bands indicates a positive result for *P.falciparum* and *P.vivax*. The presence of only one band, ″c″ within the result window indicates a negative result, as manufacturer's instructions (Rapid Malaria pf/pv Antigen Test).

Buffy coat concentration technique:

Glass test tubes with EDTA anticoagulated venous blood were centrifuged at medium speed for about 15 minutes, by small plastic pipette the supernatant plasma above the buffy coat layer was removed and discarded. The buffy coat layer and red cells immediately below it was transferred to one end of slide and mixed (thick film), by spreader made an evenly spread thin preparation. The preparations were allowed to air dry, fixed

with absolute methanol (just for thin films), stained with 10% Giemsa stain, allowed to air dry and examined under microscope first with 40x objective and then with 100x objective [(8)].

Data analysis:

Results obtained were analyzed by the computerized program of statistical package of social science (SPSS) version 11.5. Frequencies mean and Chi-squire test were used. Then data were presented in figures and tables.

Sensitivity of techniques:

Sensitivity was calculated as described by Kocharekar *et al.* [(9)]:

TP/(TP+FN) x 100%

TP= True positive

FN= False negative

RESULTS:

The study was conducted on 200 subject, 92 (46%) were males and 108 (54%) were females in table (1). The age between 1-65 years old, the mean age was 27±18 years old. Study subjects were divided into 5 age groups as follow: 1-15, 16-25, 26-35, 36-45 and 46-65 years old, the frequency of each age group was 77 (38.5%), 25 (12.5%), 32 (16%), 30 (15%) and 36 (18%) respectively in table (2). Out of 200 blood samples, 20 (10%), 25 (12.5%) and 20 (10%) were positive for *P.falciparum* by using stained blood film, ICT and buffy coat concentration technique respectively. And out of 200 blood samples, 4 (2%) were positive for *P.vivax* by using the three methods in table (3). Out of 24 positive blood samples, 13 (6.5%) were positive males and 11 (5.5%) were positive females. The relationship between malaria and gender was insignificance (p=0.392) in figure (1). The prevalence of malaria were, 12 (6%), 2 (1%), 2 (1%), 4 (2%) and 4 (2%) in age groups between 1-15 years, 16-25, 26-35, 36-45

and 46-65 respectively in table (4). When blood films compared with ICT, 23(11.5%) were positive by two methods, while 1(0.5%) blood sample was positive by blood film and negative by ICT, 6(3%) were positive by ICT and negative by blood film. While blood film and buffy coat concentration techniques showed the similar results in table (5). Sensitivity of blood films and buffy coat concentration technique was 100%, while ICT sensitivity was 96% in table (6). Out of 24 positive cases, 6(3%) presented as (+) for *P.falciparum*, 4(2%) and 1(0.5%) presented as (++) for *P.falciparum* and *P.vivax* respectively, 7(3.5%) and 2(1%) presented as (+++) for *P.falciparum* and *P.vivax* respectively, 3(1.5%) and 1(0.5%) presented as (++++) for *P.falciparum* and *P.vivax* respectively in figure (2). Out of 24 positive cases, *P.falciparum* stages were 16(8%) trophozoites, 3(1.5%) gametocytes and 1(0.5%) were trophozoites and gametocyte. *P.vivax* stages were 3(1.5%) trophozoitse and gametocyte and 1 (0.5%) were trophozoites, schizonts and gametocytes. The relationship between species and stages were significance (p=0.000) in figure (3). Out of 29 positive cases by using ICT, 7 (3.5%), 1(.5%) and18 (9%) were previously infected in the time period of about 1-30 day, 31-60 day and more than yaer respectively, while 3(1.5%) were not infected previously. Relationship between ICT positive tests and duration of previous infection was statistically significant (p=0.000) in figure (4). Out of 24(12%) positive cases by blood films, 20 (10%) were treated previously while, 4(2%) were not treated previously. From 20 (10%) treated cases, 12 (6%), 7(3.5%), 1(0.5%) were previously treated by artemether, artesunate, artemether+primaquine respectively. The relationship between malaria and previous treatment was significance (p=0.030) in figure (5).

Table (1): Frequency of study subjects according to gender

Gender	Frequency	Percentage (%)
Male	92	46%
Female	108	54%
Total	200	100%

Table (2): Frequency of study subjects according to age groups

Age groups (years)	Frequency	Percentage (%)
1-15	77	38.5%
16-25	25	12.5%
26-35	32	16%
36-45	30	15%
46-65	36	18%
Total	200	100%

Table (3): Prevalence of malaria by using blood films, ICT and buffy coat concentration technique

Techniques	***P.falciparum*** **(+ve)**	***P.vivax*** **(+ve)**
Blood films	20	4
ICT	25	4
Buffy coat technique	20	4

p=0.000

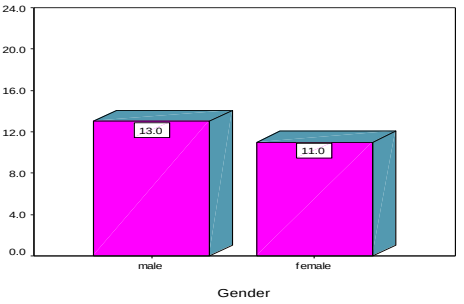

Figure (1): The overall prevalence of malaria by using blood films according to gender

Table (4): The overall prevalence of malaria by using blood films according to age groups

Age groups (years)	Blood Films		Total
	Positive	Negative	
1-15	12	65	77
16-25	2	23	25
26-35	2	30	32
36-45	4	26	30

46-65	4	32	36
Total	24	176	200

p=0.664

Table (5): Relationship between the three different methods in detection of malaria parasites

		Blood films		Total	p value
		Positive	Negative		
ICT	Positive	23	6	29	p=0.000
	Negative	1	170	171	
Total		24	176	200	
Buffy coat Technique	Positive	24	0	24	p=0.000
	Negative	0	176	176	
Total		24	176	200	

Table (6): Sensitivity of different techniques in malaria detection

Techniques	No. of positive	No. detected by the technique	Sensitivity (%)
Blood films	24	24	100%
Buffy technique	24	24	100%
ICT	24	29	96%

Table (7): Relationship between malaria and fever

		Fever		Total
		Yes	No	
B.F for Malaria	Positive	24	0	24
	Negative	158	18	176
Total		182	18	200

p=0.101

Table (8): Relationship between malaria and headache

		Headache		Total
		Yes	No	
B.F for Malaria	Positive	18	6	24
	Negative	131	45	176
Total		149	51	200

p=0.952

Table (9): Relationship between malaria and vomiting

		Vomiting		Total
		Yes	No	
B.F for Malaria	Positive	21	3	24
	Negative	66	110	176

Total	87	113	200

p=0.000

Table (10): Relationship between malaria and diarrhea

		Diarrhea		Total
		Yes	No	
B.F for Malaria	Positive	2	22	24
	Negative	25	151	176
Total		27	173	200

p=0.430

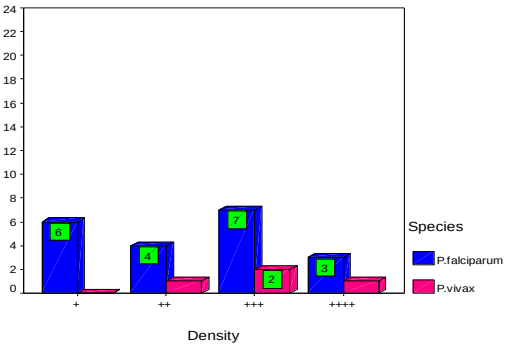

Figure (2): Intensity of malaria parasites

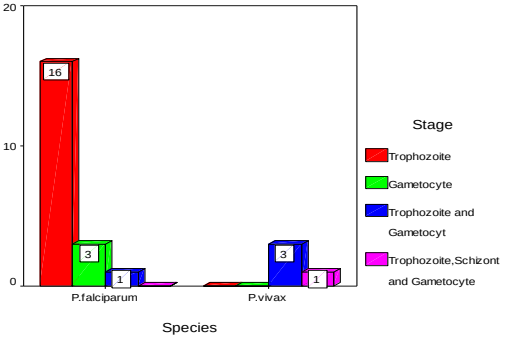

Figure (3): The overall prevalence of malaria according to stages

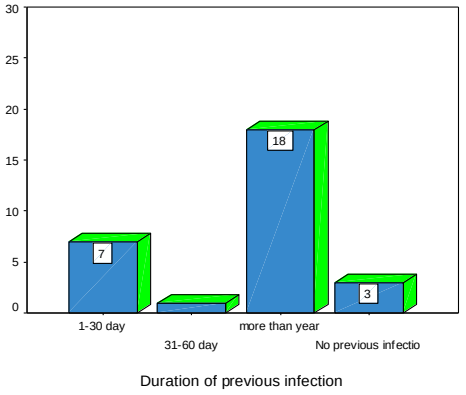

Figure (4): Prevalence of malaria according to duration of previous infection

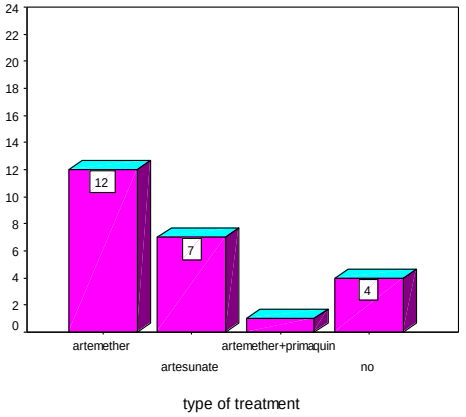

Figure (5): Prevalence of malaria according to types of previous treatment

DISCUSSION:

Malaria continues to haunt and taunt mankind. More than a century after identification of the causative parasites, and more than half a century after finding effective drugs and insecticides, the disease as old as humanity itself, affect more than 500 million and kill more than 3 million people every year [(10)]. The earliest symptoms of malaria are very non-specific and

variable. Hence there is difficult to clinically diagnose malaria but the treatment has to be started immediately in order to avoid complications. Therefore precise laboratory diagnosis and species identification is essential [(11)]. The present study was carried out on 200 blood samples collected from patients with clinical symptoms of malaria at Medical Military Hospital-Khartoum State. Out of 200 blood samples, 108(54%) were females, while 92(46%) were males with ratio of 1.2:1, this finding was in contrast to the Rashmi *et al.* findings [(12)]. The present study showed that the prevalence of malaria in the study area was 24(12%), which was more in males (6.5%) than in females (5.5%), these findings in agreement with Rashmi *et al.* findings [(12)], while the relationship between malaria and gender was insignificance (p=0.392). The prevalence was high (6%) in the age group 1-15 years old, due to their lack of efficient immunological response against the infection. The relationship between age and malaria was found to be statistically insignificant (p=0.664), it means that the infection is not affected by the age. Out of 24 positive cases, 20(10%) were positive for *P.falciparum*, 4(2%) were positive for *P.vivax* which indicates that *P.falciparum* is predominant species than *P.vivax* in the study area. These results were similar to results obtained by Medhi *et al.* [(13)]. The prevalence of malaria according to fever was 24(12%) (p=0.101), headache was 18(9%) (p=0.952), vomiting was 21(10.5%) (p=0.000), while diarrhea was 2(1%) (p=0.430). From these findings, there is no relationship between malaria infection and clinical symptoms except with vomiting. Out of 200 blood samples, 29(14.5%) were found to be positive by immunochromatographic test (ICT), while 1 case which was positive for *P.falciparum* by blood film not detected by ICT, this false negative result due to low parasitaemia. Out of 29 positive cases 6(3%) were negative by blood film and positive by ICT for *P.falciparum*, this false positive result due to antigen (HRP-II) persistent in circulation

after parasite clearance, while 23(11.5%) cases were detected by the above two methods. The result showed that, out of 200 blood samples, 24(12%) cases were positive by buffy coat concentration technique which is similar to results obtained by blood films. The results showed that the sensitivity of blood films and buffy coat concentration technique was (100%), while ICT sensitivity was (96%), a similar study done by Binesh *et al.* [14], showed sensitivity of (97.77%), (80.76) and (97.10%) for blood films, buffy coat concentration technique and ICT respectively. Sensitivity of blood films and buffy coat concentration technique was slightly higher in the present study, while ICT sensitivity was slightly lower in the present study. The present study indicated that buffy coat concentration technique provides a reliable, quick, easily mastered method for diagnosis of malaria. The method is useful in laboratories in endemic areas where parasite level is low, so it concentrated the low number of parasites. The present study showed that the antigen detection test had lower accuracy than blood films and buffy coat concentration technique. These findings were similar to findings obtained by Salmani *et al.* [11], and the gold standard for laboratory diagnosis is microscopic examination of thick and thin blood films, this result was agreed with Binesh *et al.* study [14].

CONCLUSION:

This study concluded that the prevalence rate of malaria was (12%) in the study area. Blood films examination is the gold standard for malaria diagnosis. Buffy coat concentration technique method was provided a reliable, quick, accurate method for diagnosis of malaria while ICT test showed less accuracy in malaria diagnosis. *P. falciparum* is the predominant species in the study area. Most affected age group was the group between 1-15 years.

REFERENCES:

1. Singh, N., Shukla, M. M., Shukla, M. K., Mehra, R. K., Sharma, S., Bharti, P. K., Singh, M. P., Singh, A. and Gunasekar, A. Field and laboratory comparative evaluation of rapid malaria diagnostic test versus traditional and molecular techniques in India. *Malaria J*. 2010; 9:191.
2. Greenwood, D., Barer, M., Slack, R. and Irving, W. Medical Microbiology. 18th edition, China. 2012; 642.
3. Lamb, T. J. Immunity to Parasitic Infection. 1st edition, New Delhi- India. 2012; 91-104.
4. National institute for allergy and infectious diseases (NIH). Malaria quick facts. 2010; available at: http://www.niaid.nih.gov/topics/Malaria/understandingMalaria/Pages/facts.aspx
5. John, T. and Petri, W. J. Markel and Voges Medical Parasitology. 9th edition, United States. 2006; 79-92.
6. World health organization (WHO). Sudan Malaria control and elimination. 2015; available at: http://www.emro.who.int/sdn/programmes/malaria-sudan.html
7. Ministry of Human Development and Labour. Location and area of Khartoum State. 2015; available at: http://www.mhd.gov.sd/english/index.php?option=com_content&view=article&id=248&Itemid=88
8. Cheesbrough, M. Medical Laboratory Manual for Tropical Countries. 2nd edition, Great Britain. 1987; 244-247.
9. Kocharekar, M. M., Sarkar, S. S. and Dasgupta, D. Comparative Study of Modified Quantitative Buffy Coat and Two Rapid Tests in Comparison with Peripheral Blood Smear in Malaria Diagnosis in Mumbai, India. *Journal of Parasitology Research*. 2014; 2014:7.
10. Mehta, D. and Desai, N. Laboratory Diagnosis of Malaria-Various Method and its Comparison. *NJIRM*. 2013; 4(3):138-143.

11. Salmani, M. P., Mindolli, P. B. and Peerapur, P. V. Comparative Study of Peripheral Blood Smear, QBC and Antigen Detection in Malaria Diagnosis. *JCDR*. 2011; 5(5):967-969.

12. Rashmi, R., Ambica, R. and Nagarathnamma, T. A comparative Study of Blood Smear, Quantitative Buffy Coat and Antigen Detection for Diagnosis of Malaria. *International Journal of Current Research*. 2015; 7(04):14490-14495.

13. Medhi, P., Dowerah, S and Dutta, A. Evaluation of Quantitative Buffy Coat Technique in the Diagnosis of Malaria: A Study from North East India. *Jemds*. 2015; 4(78):13560-13564.

14. Binesh lal, Y., Jayakumar, S., Kalyani, M., Mathew, R., Shameem Banu, A. S. and Dhinesh, R. Correlation of Quantitative Buffy Coat, Blood smear and Antigen Detection in Diagnosing Malarial Infection. *JCDR*. 2011; 5(5):961-963.