International Journal of Academic Multidisciplinary Research (IJAMR)
ISSN: 2643-9670
Vol. 4, Issue 5, May – 2020, Pages: 98-103

Laboratory Comparative Evaluation of Rapid Malaria Diagnostic Test Versus Microscopy in Gezira State- Sudan

*** Tayseer Elamin Mohamed Elfaki**

Department of Parasitology and Medical Entomology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan

***Corresponding Author**: Tayseer Elamin Mohamed Elfaki, *Email*: tayseeralfaki5@gmail.com

Abstract: *The main aim of this study was to evaluate the immunochromatographic test versus microscopy for the detection of malaria in Gezira State- Sudan. A cross- sectional study was carried out during the period between December 2008 to January 2009. A total number of 250 students (age between 6-16 years old, mean age was 11 years) were included in this study. Male: female ratio was 71:179 (28.4% and 71.6% respectively). Blood samples were taken from all subjects. Clinical and parasitological data were obtained and recorded. Out of 250 blood samples, 71 (28%) were positive for P.falciparum by using Giemsa's stained blood films. Moreover, out of 250 blood samples, 80 (32%) were found to be positive when examined by immuno chromatogrophic test (ICT). In this study no difference in gender and age was found in relation to malaria infection. The study reflected that all the positive cases were P.falciparum which indicates that P.falciparum is predominant species in the study area. The current study showed that there was no relationship between malaria infection and clinical symptoms (fever, headache, vomiting and diarrhea). This study indicated that the study area is highly endemic for malaria and the prevalence rate of malaria infection is reflecting high. On the other hand, the study demonstrated that very high sensitivity (100%) for the ICT for malaria diagnosis.*

Keywords— Laboratory comparative evaluation; *P.falciparum*; ICT; Gezira state

1. INTRODUCTION

Malaria is the most important of the life threatening protozoan disease, which is responsible for at least 750,000 deaths a year, mostly in young children in Africa [1]. 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 result in death. Most of the fatalities are in children under the age of five years old and pregnant women [2]. 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% [3]. 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 [3]. 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 [3]. 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 [3]. Several rapid diagnostic tests (RDTs) are available, which are fast, reliable and simple to use and can detect *P.falciparum* and non-*falciparum* infections or both [4]. RDTs are based on the detection of antigens derived from malaria patients in lysed blood, using immunochromatographic methods. Most frequently they employ a dipstick or test strip bearing monoclonal antibodies directed against the target parasite antigens [5]. The objective of the present study was to evaluate the immunochromatographic test versus microscopy for the detection of malaria in Gezira State- Sudan.

2. Materials and methods

2.1 Study design:

It is a cross-sectional study.

2.2 Study area:

This study was carried out in El Genaid area, Gezira State. The area is located on the East bank of the Blue Nile. The study area composed of El Genaid irrigated Scheme and El Genaid sugar factory. The area is surrounded by big and small irrigation canals. The area is considered to be endemic for malaria.

2.3 Study population:

For the purpose of this study two primary schools were selected (ELtadamon primary school for girls and ELtadamon primary school for boys). A total of 250 children (age range 6-16 year old) were included in this study during the period between December 2008 and January 2009. After informed consent was obtained all children included in this study have agreed to participate in the study.

2.4 Sample size:

A total of 250 blood samples were examined.

2.5 Data collection:

The primary data were collected by using self-administrated per-coded questionnaire which was specifically designed to obtain information that helped in the study.

3. Methods

3.1 Collection of blood samples:

With cotton wool dipped in 70% ethanol, the tip of the third finger was cleaned. With sterile lancet the finger was pricked firmly and rapidly. The first drop of blood was wiped out. Single drop of blood was placed on the middle of a clean slide for thin smear. Three drops were placed in apart from the first drop for thick smear. Blood samples for Immuno Chromatographic Technique (ICT) were collected in capillary tube.

3.2 Preparation of blood films:

To make thick smear, the collected blood was stirred with a corner of a clean glass slide until an appropriate thickness was obtained. To make the thin smear, the edge of spreader was placed just in-front of the drop of blood. Then it was drawn back until it touches the drop of blood. The blood was allowed to run along the edge of the spreader. The spreader was then pushed to the other end of the slide with a smooth movement. The slide was then allowed to dry.

3.3 Staining of blood films:

All thin and thick blood films were stained using Giemsa stain.

(i) Only thin films were fixed with methanol for (1-2 minutes).

(ii) The slides were covered with 10% Giemsa solution for 10 minutes. All slides were washed using clean water and allowed to dry by air.

3.4 Examination of blood films:

The slides were examined using light microscope (Olympus x100 oil immersion lens) the number of parasites were counted and reported by using the following grading (according to WHO, 2014) [6]:

1- 10 parasites per 100 thick- film fields +
11- 100 parasites per 100 thick- film fields + +
1- 10 parasites per thick- film field + + +
More than 10 parasites per thick- film field + + + +

3.5 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).

3.6 Data analysis:

All information and data were entered in Computer. Data were analyzed and tested by Chi Square Test and descriptive statistics using statistical package of social science (SPSS) version 11.5 for windows.

3.7 Sensitivity and specificity of ICT:

Sensitivity and specificity were calculated by Zhu *et al.* (2010) [7] as follow:-

Sensitivity= TP/ (TP+FN) ×100%
Specificity= TN/ (TN+FP) ×100%
TP= True positive
FN= False negative
TN= True negative
FP= False positive

4. Results

The age of the study subjects included in the present study ranges between 6-16 years, mean age was 11years. Male: Female ratio was 71:179 (1:2.5) (28.4% and 71.6% respectively) (table 1). Seventy one out of 250 of the study subjects were found to be harboring *P.falciparum* parasite. The malaria blood film point prevalence was 28% (figure 1). The degree of malaria parasitaemia among the study subjects was shown in (table 2). The prevalence of *P. falciparum* malaria was increased using ICT method. Eighty of the study subjects (32%) were found to be positive (figure 2). The relationship between malaria and headache was shown in (table 3). The relationship between malaria and fever was shown in (table 4). The relationship between malaria and vomiting was shown in (table 5). The relationship between malaria and diarrhea was shown in (table 6). When blood films compared with ICT, 71 (28.4%) were positive by two methods, while there was no blood sample was positive by blood film and negative by ICT and 9 (3.6%) were positive by ICT and negative by blood film (table 7). So, the sensitivity and specificity of ICT according to formula mentioned in materials and methods were 100.0% and 95% respectively.

Table 1: Frequency of gender

Gender	Frequency	Percentage (%)
Male	71	28.4
Female	179	71.6
Total	250	100.0

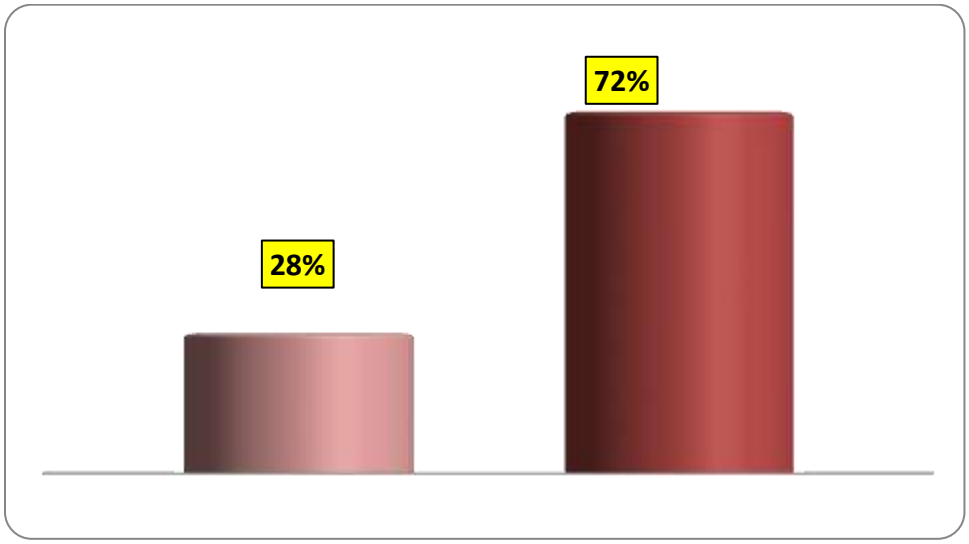

Figure 1: The malaria parasite point prevalence in the study area

Table 2: The degree of malaria parasitaemia among the study subjects

Parasitaemia	Frequency	Percentage
Severe	24	9.6%
Moderate	10	4.0%
Mild	36	14.4%
Negative	180	72.0%
Total	250	100.0%

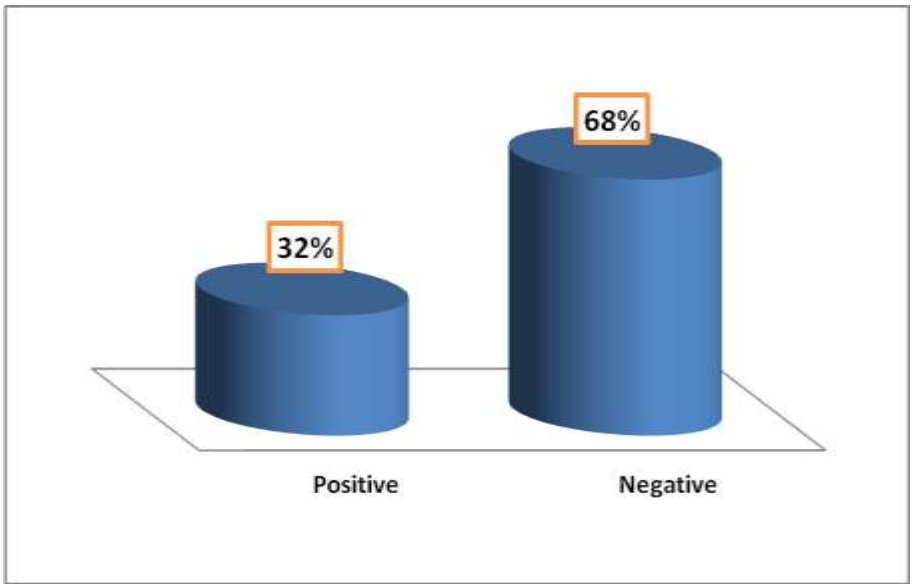

Figure 2: The ICT *P. falciparum* point prevalence in the study area

Table 3: Relationship between malaria and headache

		Headache		Total
		Yes	No	
B.F for malaria	+ve	59	11	70
	-ve	13	167	180
Total		72	178	250

Table 4: Relationship between malaria and fever

		Fever		Total
		Yes	No	
B.F for malaria	+ve	58	12	70
	-ve	11	169	180
Total		69	181	250

Table 5: Relationship between malaria and vomiting

		Vomiting		Total
		Yes	No	
B.F for malaria	+ve	46	24	70
	-ve	9	171	180
Total		55	195	250

Table 6: Relationship between malaria and diarrhea

	Diarrhea	Total

		Yes	No	
B.F for malaria	+Ve	37	33	70
	-Ve	28	152	180
Total		65	185	250

Table 7: Relationship between the blood film and ICT in detection of malaria parasites

		Blood films		Total	P value
		Positive	Negative		
ICT	Positive	71	9	80	p=0.000
	Negative	0	170	170	
Total		71	179	250	

5. Discussion

The present study was carried out on 250 blood samples collected from children, 179 (71.6%) were females, while 71 (28.4) were males with ratio of 2.5:1, this finding was in agreement with finding of Hamza *et al*. (2016) [8] and in disagreement with finding of Rashmi *et al.* (2015) [9]. The current study showed that the prevalence rate of malaria as confirmed by presence of parasite in Giemsa's stained blood smears was 28% while positivity was increased (32%) when using ICT as a diagnostic technique. This might be explained by the fact that ICT is detected Histidine Rich Protein2 (HRP2) which remains circulating in the blood for a period of time after treatment. In this study no difference in gender and age was found in relation to malaria infection. These findings were in agreement with the findings obtained by Hamza *et al*. (2016) [8]. The high prevalence of malaria in the study area is attitude to the fact that the area is considered to be one of the major sugar plants in Sudan and the irrigation depends on cannal systems and this provides a suitable environment for malaria transmission. All the positive cases were *P.falciparum* which indicates that *P.falciparum* is predominant species in the study area. These results were similar to results obtained by Medhi *et al.* (2015) [10] and Hamza *et al*. (2016) [8]. The current study showed that there was no relationship between malaria infection and clinical symptoms (fever, headache, vomiting and diarrhea), these findings were in agreement with the findings obtained by Hamza *et al*. (2016) [8]. The results showed that the sensitivity and specificity of ICT were 100% and 95% respectively, which were higher than a 96% and 97.1% sensitivity obtained by Hamza *et al*. (2016) [8] and Binesh *et al.* (2011) [11] respectively.

6. Conclusion

The present study concluded that**,** the prevalence rate of malaria in the study area was 28% and 32% by using microscopy and ICT respectively. Sensitivity of the ICT was very high (100%). *P. falciparum* is the predominant species in the study area.

References

[1]- Greenwood, D., Barer, M., Slack, R. and Irving, W. (2012). Medical Microbiology. 18th edition, China. 642.
[2]- Lamb, T. J. (2012). Immunity to Parasitic Infection. 1st edition, New Delhi- India. 91-104.
[3]- World health organization (WHO) (2015). Sudan Malaria control and elimination. Available at: http://www.emro.who.int/sdn/programmes/malaria-sudan.html.
[4]- Singh, N., Shukla, M. M., Shukla, M. K., Mehra, R. K., Sharma, S., Bharti, P. K., Singh, M. P., Singh, A. and Gunasekar, A. (2010). Field and laboratory comparative evaluation of rapid malaria diagnostic test versus traditional and molecular techniques in India. *Malaria J*, 9:191.
[5]- Arora, D. R. and Arora, B. B. (2010). Medical Parasitology. 3rd edition, New Delhi-India. 80-81.
[6]- World health organization (WHO) (2014). Tropical Medicine and International Health, published by John Wiley & Sons; 19 (1):7-131.
[7]- Zhu, W., Zeng, N. and Wang, N. (2010). Sensitivity, specificity, accuracy, associated confidence interval and ROC analysis with practical SAS implementations. *Health care and Life Sciences*, 19:67.
[8]- Hamza, S. E. S., Elfaki, T. E. M. and Abd Alla, A. B. (2016). Evaluation of Malaria Diagnostic Methods in Medical Military Hospital, Khartoum State, Sudan. *European Academic Research*; (3)10:21-11356.
[9]- Rashmi, R., Ambica, R. and Nagarathnamma, T. (2015). A comparative Study of Blood Smear, Quantitative Buffy Coat and Antigen Detection for Diagnosis of Malaria. *International Journal of Current Research*, 7(04):14490-14495.
[10]- Medhi, P., Dowerah, S and Dutta, A. (2015). Evaluation of Quantitative Buffy Coat Technique in the Diagnosis of Malaria: A Study from North East India. *Jemds*, 4(78):13560-13564.

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