Journal of the Egyptian Society of Parasitology, Vol. 50, No.3, December 2020
J. Egypt. Soc. Parasitol. (JESP), 50(3), 2020: 483-487
(Online: 2090-2549)

MALARIA AMONG IMMIGRANT'S WORKFORCE RETURNING FROM ENDEMIC DISTRICTS IN ALWOSTA CITY, EGYPT

By
ENAS Y. ABU-SAREA[1], MOUSA A. M. ISMAEL[2], RAAFAT M. SHAAPAN[3], SAMAH S. ABDEL GAWAD[1] AND MARWA A. GHIETH[1]
Department of Medical Parasitology, Faculties of Medicine, Beni-Suef University[1] and Cairo University[2], and Department of Zoonotic Diseases, National Research Centre[3], El-Tahrir Street, Dokki, Giza, Egypt. (*Correspondence: marwaghieth@yahoo.com)

Abstract

This research aimed to encounter rapid diagnostic test (RDTs) efficacy to distinguish malaria within patients with positive history to fever and/or travelling to endemic municipality. All over a year, blood samples were amassed from patients visited different laboratories. Both thin, thick blood films stained with Geimsa and rapid tests were used to diagnose malaria. Malaria was detected by microscopy and RDTs (0.5%, & 0.9%, respectively) of patients. RDTs yielded 100% sensitivity and 98.5% specificity. Samples detected by microscopy were also detected by RDTs. All diagnosed malaria patients had history of travelling to Sudan or Yemen. RDTs proved confirmatory tool to catch malaria suspected cases with fever history. Travelers from Sudan and Yemen should be checked for malaria.

Key words: Egypt, Patients, Malaria, Rapid diagnostic tests, Microscopy.

Introduction

Malaria is the most crucial parasitic disease worldwide despite the efforts done to eliminate infection, but treatment and control programs were effective in low endemic areas (Baird, 2010). About 200 million malaria infections and 400,000 deaths were reported (WHO, 2016).

The convenient of malaria diagnosis helped maintaining proper control and treatment, using microscopy, the still gold standard, cheap, differentiated species and estimated infection density (Chiodini, 2014). RDTs had developed as simple noninvasive aiding method with satisfied sensitivity & acceptable specificity to detect malarial antigen among fresh and stored blood samples (Choto *et al,* 2015; Zaki and Madkhali, 2016). WHO used RDTs and microscopy among clinically suspected cases initial to treatment (Fransisca *et al,* 2015). To depend on clinical and laboratory diagnosis was more reliable (Kyabayinze *et al,* 2008).

The study aimed to test detection of malaria among patients with fever history in Al wosta City, Beni-Suef Governorate and to evaluate efficacy of used diagnostic tests.

Material and Methods

Study population: A cross sectional study of 732 patients attended three medical laboratories at Alwosta City, Beni-Suef Governorate. Samples were collected over a year, from August 2015 to 2016. Patients were selected on behalf of having fever history and/or travelling to malaria endemic regions. Patients from all ages and both sexes were included. A questionnaire was fulfilled included age, sex, residence, history of travelling to malaria endemic regions, and fever.

Ethical consideration: The study was done in accordance with ethics of the Helsinki Declaration for sample collections. Patients were informed about the study aim and were free to reject collaboration

Blood samples were collected from all patients and tested for malaria parasites using finger prick capillary blood for thick and thin blood films processing's. Films were dried and fixed with absolute methanol and covered with foil till examination. To detect malarial antigen using RDTs, blood samples were obtained on EDTA and stored at -20^{o}C RDTs processing.

Microscopic examination of blood samples: Stained films with 10% diluted Giemsa were prepared and examined for malaria with species identification (Warhurst and Williams, 1996). Positive results were considered if one malaria stage was detected, negative results were considered if high po-

wer oil immersion showed no parasites in the field.

Detection of malaria antigen by RDTs: Processing was done using *in vitro* malaria rapid test cassette, a qualitative immune-chromatoghraphic test to detect *Plasmodium* species antigens in the whole blood after the manufacturer's instructions.

Detection of *P. falciparum* specific histidine rich protein 2 (PfHRP-2) antigens and plasmodia specific lactate dehydrogenase (LDH) occurred by RIDA®Quick Malaria (N7006, Germany).

Statistical analysis: Data were analyzed using SPSS software version 20, as (M±SD).

All variables were presented as frequency, and comparison was done using Chi-square & t-test. P value > 0.05 was considered insignificant. RDTs sensitivity and specificity were calculated with microscopy as the gold standard. Positive malaria antigen cases were considered the dependent variable to estimate risk factors for infection.

Results

Descriptive data: Ages was 36.59±17.2, male represented 65.4% and 34.6% were females, and from rural areas (63.7%)

Microscopic examination showed 4 positive samples (0.5%) distributed as, *P. vivax, P. falciparum,* mixed *P. falciparum & P. vivax* infections (0.5%, 0.25%, & 0.25%, respectively).

Malaria antigen was detected in 7 cases (0.9%). All microscopically positive samples were positive for antigens. They traveled to Sudan (71.4%) or Yemen (28.6%). Considering microscopy as a gold standard, the antigen detection showed 100% sensitivity and 98.5% specificity. Patients with positive antigen were males (71.4%) from rural areas (57.1%) with age group >30-40 (42.8%). All patients gave fever history and traveled to either Sudan or Yemen. None of sex, age groups or residence was risk factor for malaria antigen. Details were given in tables (1, 2, 3 & 4).

Table 1: Sociodemographic characters of all patients

Variables		n= 732 (%)
Sex	Male	479 (65.4)
	Female	253 (34.6)
Age (M ± SD)		36.59±17.2
Residence	Rural	466 (63.7)
	Urban	266 (36.3)
Detection of Malaria	Microscopy	4 (0.5)
	Antigen detection	7 (0.9)
History of travelling*	Yes	7 (0.9)
	No	725 (99.1)

Table 2: Microscopy versus antigen detection of Malaria (n=732)

		Antigen detection (n=7)		
		+ve	–ve	Total
Microscopy (n=4)	*P. falciparum*	1	0	1
	P. vivax	2	0	2
	P. falciparum & P. vivax	1	0	1
	+ve	4	0	4
	-ve	3	725	728
	Total	7	725	732

Table 3: Kappa agreement of antigen detection

	Microscopy	(κ)* Interpretation
Sensitivity	100%	< 0: Poor agreement
Specificity	98.5%	0.01 – 0.20: Slight agreement
PPV	57.14%	0.21 – 0.40: Fair agreement
NPV	100%	0.41 – 0.60: Moderate agreement
Accuracy	99.2%	0.61 – 0.80: Substantial agreement
Kappa (κ)*	0.67	0.81 – 1.00: Almost perfect agreement

Table 4: Characteristics of patients with positive antigen detection

Variables		Malaria +ve antigen n=7 (%)	Malaria –ve antigen n=725 (%)	Total n=732 (%)	*P* value
Sex	Male	5 (71.4)	474 (65.3)	479 (65.4)	0.41
	Female	2 (28.6%)	251 (34.7)	253 (34.6)	
Age (Mean ±SD)		33.3±15.4	35.6±11	36.5±17.2	0.71
Age group: (>20-30)		2 (28.6)	214 (29.5)	216 (29.5)	
: (>30-40)		3 (42.8)	318 (43.8)	321 (43.8)	0.3
: (>40-50)		2 (28.6)	171 (23.5)	173 (23.6)	
: (>50-60)		0	22 (3.03)	22 (3)	
Residence	Rural	4 (57.1)	462 (63.7)	466 (63.7)	0.5
	Urban	3 (42.9)	263 (36.2)	266 (36.3)	

Discussion

In the ancient time, Egypt was a malaria endemic area even with PCR detection of infection in old mummies (Lalremruata *et al,* 2013). But, Egypt had eliminated malaria (WHO, 2012), without autochthonous focus, most reported cases were sporadic (Zaher *et al,* 2007; El-Bahnasawy *et al,* 2011), except *P. vivax* identified at Aswan Governorate were Sudanese visitors (WHO, 2014). Also, Al-Agroudi *et al.* (2018) in 3 years retrospective study of malaria in an Egyptian Fever Hospital reported 100 malaria youth patients returning back from different Sub-Sahara of African Countries. The infective malaria species were *P. falciparum* (83 cases), *P. vivax* (10 cases), *P. ovale* (1case) and mixed infections (6 cases).

In the present study, positive malaria cases by stained blood films and antigen detection were 7 with history of back from Sudan (n=5) and Yemen (n=2). Positive cases were males (71.4%) with age of 30-40 (42.8%) the age for travelling work and for commercial activity. The activity of individual travelling and immigration to endemic regions with plasmodial infection proportionally increased the risk of malaria re-introduction (Dahesh *et al,* 2009). Since 2004, Egyptians have visa free access to Sudan making travel easy for work, agriculture even political motives. It is not mandatory to get malaria prophylactic medications or mosquito protections measures on crossing borders to Sudan, making non immunized travelers at risk to catch infections where malaria and human immunodeficiency virus (HIV) were endemic (Saleh *et al,* 2019). The other 2 cases had travelled to Yemen where malaria was complicated mainly the chloroquine resistance *falciparum* with increasing risk with limited access to safe water and the ongoing internal wars (Morsy *et al,* 2019).

In the present study, positive cases (4/7) were detected by microscopy. Low parasitemia tissue sequestration of the parasite with blood clearance, suspected relapse, receiving antimalarial drugs, however no available data for receiving antimalarial drugs by the present group, that relieved parasites from the blood with maintained antigenemia (Srinivasan *et al,* 2000; Iqbal *et al,* 2004).

RDTs detected malaria earlier than microscopy, but human antibodies cross reaction, mainly the heterophile antibodies (Moody, 2002) or rheumatoid factor (Laferi *et al,* 1997. This agreed with Leslie *et al.* (2014), who reported that RDTs gave higher sensitivity than microscopy. Kamel *et al.* (2016) reported that 2/3 of *Plasmodium* antigen was missed by microscopy due to the low parasitemia.

Microscopy carries risk of false negative with low density infection and errors in species identification (Ohrt *et al,* 2002; Martín−Díaz *et al,* 2018), however it was still the gold standard by the FDA to evaluate malaria drug and vaccine efficacy (Wongsrichanalai *et al,* 2007). In non-endemic areas, RDTs confirmed infection by low microscopic parasite density detected by microscopy, explained an anti-parasitic immune response for the first exposure to infection (Zakia *et al,* 2016). Also, it did-n't criminate *falciparum* or mixed ones as nonspecific (Soliman *et al,* 2018)

The present results showed that *P. vivax, P. falciparum* followed by mixed *P. vivax* & *P. falciparum* infections were 0.5%, 0.25%, & 0.25%, respectively. At 2014, an Egypti-

an outbreak at Aswan Governorate included 20 cases (95.3%) *P. vivax* and (4.6%) a *falciparum* cases (Kandeel *et al,* 2016). But, Kamel *et al.* (2016) in El Fayoum Governorate reported *P. falciprum* in three patients' travelled to Sudan. Abo Hashim *et al.* (2017) reported imported *P. falciparum* (60%), among travelers to African Countries. Patients returned back to Egypt from Sudan recorded the commonest imported *falciparum* malaria (El-Bahnasawy *et al,* 2010). So, the strict measures should be followed in south Egypt to prevent malaria re-introduction of (Bakr *et al,* 2017).

Specific HRP-2 is the commonly Malaria antigen focused by RDTs for *P. falciparum*. Persistent HRP-2 aids in detecting low and fluctuating parasitemia (Bell *et al,* 2005). PfHRP2-based diagnostic tests were reliable for *P. falciparum* detection more sensitive than PfLDH based tests (Martín‑Díaz *et al,* 2018). Gametocytes of *Plasmodium* produce LDH antigen thus RDTs targeting LDH was still positive even after clearance of asexual stages (Miller *et al,* 2001). Using RDTs increased the frequency of malaria detection than microscopy (Mfueni *et al,* 2018), although Al-Agroudi *et al.* (2018) in Egypt found that RDTs failed to detect 4% of positive microscopic stained blood films

Conclusion

Using RDTs to confirm suspected malaria was efficient in area without microscopy facility. Sudan and Yemen are risk areas for imported malaria.

Prophylactic measures is a must for more reinforcement and collaborations of WHO Authorities with strict before travel counseling education for travelers to endemic areas to avoid spread of malaria illness.

Acknowledgements

The authors are grateful to the Scientific Research Developing Unit, Beni-Suef University for funding this research to the author (Abdel Gawad S.S), Also, appreciations to the Diagnostic & Research Unit of Parasitic Diseases (DRUP), Medical Parasitology Department, Faculty of Medicine, Beni-Suef University, for allowing samples processing.

Conflict of interest: The authors did not any conflict of interest.

References

Abo Hashim, A, Zaalouk, T, Saad, M, 2018: Imported Malaria in Egypt. Egypt. J. Hosp. Med. 67, 1:455-8.

Al-Agroudi, MA, Ahmad, GMS, Kondo, MK, Morsy, TA, 2018: Malaria situation in Egypt the last three years: Retrospective study in an Egyptian Fever Hospital. J. Egypt. Soc. Parasitol. 48, 3:635-44.

Baird, JK, 2010: Eliminating malaria all of them. Lancet 376, 9756:1883-5.

Bakr, S, Edris, S, Fattah, NSA, Ibrahim, NM, El-Khadragy, MF, 2017: Molecular screening for malaria among blood donors in a WHO claimed region of Egypt, Fayoum Governorate. Mediterr. J. Hematol. Infect. Dis. 9, 1:e2017065.

Bell, DR, Wilson, DW, Martin, LB, 2005: False-positive results of a *Plasmodium falciparum* histidine-rich protein 2-detecting malaria rapid diagnostic test due to high sensitivity in a community with fluctuating low parasite density. Am. J. Trop. Med. Hyg. 73:199-203.

Chiodini, PL, 2014: Malaria diagnostics: Now and the future. Parasitology141, 14:1873-9.

Choto, RC, Midzi, SM, Mberikunashe, J, Tshimanga, M, Gomb, NT, *et al,* 2010: Evaluation of the performance of two diagnostic assays in malaria diagnosis in Mashonaland and East Province, Zimbabwe. Open J. Epidem. 5, 03: 187-96.

Dahesh, SM, Bassiouny, HK, El-Masry, SA, 2009: Socioeconomic and environmental factors affecting malaria infection in Fayoum Governorate, Egypt. J. Egypt Soc. Parasitol. 39, 2:511-23.

El-Bahnasawy, MM, Saleh, NM, Khalil, MF, Morsy, TA, 2011: The impact of three anopheline mosquito species in Toshka, on the introduction of chloroquine resistant *P. falciparum* to Egypt. J. Egypt. Soc. Parasitol. 41, 3:573-92.

Fransisca, L, Kusnanto, JH, Satoto, TBT, Sebayang, B, Supriyanto, *et al,* 2015: Comparison of rapid diagnostic test plasmatic malaria-3, microscopy, and quantitative real time PCR for diagnoses of *Plasmodium falciparum* and *Plasmodium vivax* infections in Mimika Regency, Papua, Indonesia. Malar. J. 14, 1:103-7.

Iqbal, J, Siddique, A, Jameel, M, Hira, PR, 2004: Persistent histidine-rich protein 2, parasite lactate de-hydrogenase, and pan malarial antigen

reactivity after clearance of *Plasmodium falciparum* mono infection. J. Clin. Microbiol. 42, 9: 4237-41.

Kandeel, AA, Haggag, M, Abo El Fetouh, M, Naiel, SA, Refaey, AH, *et al,* 2016: Control of malaria outbreak due to *Plasmodium vivax* in Aswan Governorate, Egypt. EMH 22:4-10.

Kamel, MM, Attia, SS, Emam, GD, Al Sherbiny, NA, 2016: The validity of rapid malaria test and microscoopy in detecting malaria in a pre-elimination region of Egypt. Scientific 4048032/ https://doi.org/ 10.1155/4048032

Kyabayinze, DJ, Tibenderana, JK, Odong, G W, Rwakimari, JB, Counihan, H, 2008: Operational accuracy and comparative persistent antigenicity of HRP2 rapid diagnostic tests for *Plasmodium falciparum* malaria in a hyper endemic region of Uganda. Malar. J. 7, 1:221-6.

Lalremruata, A, Ball, M, Bianucci, R, Welte, B, Nerlich, AG, *et al,* 2013: Molecular identification of *falciparum* malaria and human tuberculosis co-infections in mummies from Fayum depression (Lower Egypt). PloS One 8, 4: e60307.

Laferi, H, Kandel, K, Pichler, H, 1997: False positive dipstick test for malaria. N. Engl. J. Med. 337:1635-6.

Leslie, T, Mikhail, A, Mayan, I, Cundill, B, Anwar, M, *et al,* 2014: Rapid diagnostic tests to improve treatment of malaria and other febrile illnesses: patient randomized effectiveness trial in primary care clinics in Afghanistan. BMJ 348: 3730.

Martín-Díaz, A, Rubio, JM, Herrero-Martínez, J M, Lizasoain, M, Ruiz, JM, *et al,* 2018: Study of the diagnostic accuracy of microbiological techniques in the diagnosis of malaria in the immigrant population in Madrid. Malar. J. 17, 1: 314-6.

Mfueni, E, Devleesschauwer, B, Rosas-Aguirre, A, Van-Malderen, C, Brandt, PT, *et al,* 2018: True malaria prevalence in children under five: Bayesian estimation using data of malaria household surveys from three Sub-Sahara Countries. Malar. J. 17:65.

Miller, RS, McDaniel, P, Wongsrichanalai, C, 2011: Following the course of malaria treatment by detecting parasite lactate dehydrogenase enzyme. Br. J. Haematol. 113:558-9.

Moody, A, 2002: Rapid diagnostic tests for malaria parasites. Clin. Micorbiol. Rev. 15:66-78.

Morsy, TA, Khalil, HMM, Nassar, SM, 2019: Malaria: prophylaxis is a cornerstone in management. J. Egypt. Soc. Parasitol. 49, 1:39-40

Murray, CJLC, Rosenfeld, SS, Lim, KG, Andrews, KJ, Foreman, D, *et al,* 2012: Global malaria mortality between 1980 & 2010: A systematic analysis. Lancet 379, 9814:413-31.

Ohrt, C, Purnomo, Sutamihardja, MA, Tang, D, Kain, K, 2002: Impact of microscopy error on estimates of protective efficacy in malaria-prevention trials. J. Infect. Dis. 186:540-6.

Saleh, AMA, El Nakib, MM, Malek, DMA, Morsy, TA, 2019: Mini-review on malaria and human immunodeficiency virus in Sub-Sahara Africa. J. Egypt. Soc. Parasitol. 49, 1:61-72

Srinivasan, S, Moody, AH, Chiodini, PL, 2000: Comparison of blood-film microscopy, the optimal dipstick, Rhodamine 123 fluorescence staining and PCR, for monitoring antimalarial treatment. Ann. Trop. Med. Parasitol. 94, 3:227-32.

Soliman, RH, Garcia, P, Elzagawy, SM, Hussein, B, Mayah, W, *et al,* 2018: Imported and autochthonous malaria in West Saudi Arabia: results from a reference hospital. Malar. J. 17, 1: 286.

Warhurst, DC, Williams, JE. ACP, 1996: Broadsheet no 148: Laboratory diagnosis of malaria. J. Clin. Pathol. 49, 7:533-8.

WHO, 2012: Global Malaria Program: Good practices for selecting and procuring rapid diagnostic tests for malaria. Geneva, Switzerland.

WHO, 2014: Vector-borne diseases, Geneva.

WHO, 2016: WHO-scales-up-response-for-increase-in-malaria in Yemen. http://www. emro. who. int/yem/ yemen-news/ who-scales-up-response -for-increase-in-malaria-in-yemen.html

Wongsrichanalai, C, Barcus, MJ, Muth, S, Sutamihardja, A, Wernsdorfer, WH, 2007: A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT). Am. J. Trop. Med. Hyg. 77:119-27.

Zaher, T, Ahmadi, M, Ibrahim, A, El-Bahnasawy, M, Gouda, H, *et al,* 2007: Malaria in Egypt, Saudi Arabia and Yemen: A clinical pilot study. J. Egypt. Soc. Parasitol. 37, 3:969-76.

Zaki, WM, Madkhali, AM, 2016: Accuracy of immunochromatography diagnostic test versus microscopy in the diagnosis of malaria among clinically suspected patients in Jazan area, KSA. Parasitol. Unit. J. 9, 2:95-102.