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Investigation of Sensitivity of Rapid Diagnosis Tests in Patients with Suspected Malaria

Sıtma Şüpheli Hastalarda Hızlı Tanı Kitlerinin Duyarlılığının Araştırılması

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ABSTRACT

Objective: Malaria has been eradicated in Türkiye as of 2010, but there are imported cases. In this study, we aimed to compare the diagnostic value of two rapid tests; SD Bioline Malaria Ag Pf/Pan (SD-Pf/Pan) and SD Bioline Malaria Ag Pf/Pv (SD-Pf/Pv) with microscopy and real time-polymerase chain reaction (RT-PCR).

Methods: Blood samples were taken from all participants. Thick drop smears were prepared. Thick drop smears were examined for malaria positive/negative distinction under the light microscopy. Then, two rapid diagnostic tests (SD-Pf/Pan and SD-Pf/Pv) were performed. After DNA extraction from blood samples, RT-PCR was typed. The data were evaluated with SPSS 21 program of statistics.

Results: A total of 30 cases out of 66 suspected malaria cases were detected as positive with microscopy and RT-PCR. Twenty-seven patients were found positive with both SD-Pf/Pan and SD-Pf/Pv tests. Based on the microscopic results as a reference method, SD-Pf/Pan and SD-Pf/Pv rapid diagnostic tests had a 90% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 92.86% negative predictive value (NPV). Based on the RT-PCR results as a reference method, for detection of *P. falciparum,* both tests had a 95.65% sensitivity, 100% specificity, 100% PPV, and 88.89% NPV. Moreover, while SD-Pf/Pv had a sensitivity, specificity, PPV, and NPV of 100% in detection of *P. vivax*; SD-Pf/Pan has a 77.78% sensitivity of, 61.90% specificity of, 46.67% PPV, and 86.67% NPV SD-Pf/Pan for detection of PAN.

Conclusion: As a result, high sensitivity and specificity were detected in both kits in the diagnosis of malaria infections caused by *P. falciparum* and *P. vivax*. Rapid diagnostic tests can be used safely in diagnosis however the diagnosis should be supported by microscopy and RT-PCR methods when they are applicable.

Keywords: Malaria, rapid diagnosis test, real-time polymerase chain reaction, Hatay

ÖZ

Amaç: Sıtma, 2010'dan itibaren Türkiye'de eradike edilmiş ancak importe olgular görülmektedir. Çalışmada, SD Bioline Malaria Ag P.f/Pan (SD-Pf/Pan) ile SD Bioline Malaria Ag P.f/P.v (SD-Pf/Pv) olmak üzere iki hızlı tanı kiti ile mikroskop inceleme ve gerçek zamanlı-polimeraz zincir reaksiyonu (GZ-PZR) yönteminin karşılaştırılarak hızlı tanı testlerinin tanı değerinin ortaya konması amaçlanmıştır.

Yöntemler: Tüm katılımcılardan kan örnekleri alındı. Kalın damla yaymaları hazırlandı. Işık mikroskobu altında sıtma pozitif/negatif ayrımı açısından kalın damla yaymaları incelendi. Daha sonra SD-Pf/Pan ve SD-Pf/Pv olmak üzere iki hızlı tanı testi ile çalışıldı. Kan örneklerinden DNA ekstraksiyonu yapıldı ve GZ-PZR ile tiplendirildi. Veriler SPSS 21 programı ile istatistiksel olarak değerlendirildi.

Bulgular: Altmış altı sıtma şüpheli hastadan 30'unun mikroskop inceleme yöntemi ve GZ-PZR ile pozitif olduğu belirlendi. Yirmi yedi hastada hem SD-Pf/Pan hem de SD-Pf/Pv testlerinde pozitif bant elde edildi. Referans olarak mikroskop inceleme yöntemi baz alındığında SD-Pf/Pan ve SD-Pf/Pv hızlı tanı testleri %90 duyarlılığa, %100 özgüllüğe, %100 pozitif prediktif değerine (PPV) ve %92,86 negatif prediktif değerine (NPV) sahipti. Referans olarak GZ-PZR sonuçlarına göre, *P. falciparum* tespiti için her iki testin duyarlılığı %95,65, özgüllüğü %100, PPV %100 ve NPV %88,89'du. Ayrıca SD-Pf/Pv'nin *P. vivax* tespitinde duyarlılığı, özgüllüğü, PPV ve NPV'i %100 iken; SD-Pf/Pan'ın, PAN tespiti için duyarlılığı %77,78, özgüllüğü %61,90, PPV %46,67 ve NPV %86,67 olarak bulundu.

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Türkiye PARAZİTOLOJİ Dergisi

Sonuç: Sonuç olarak *P. falciparum* ve *P. vivax*'ın neden olduğu sıtma enfeksiyonlarının tanısında her iki kitin de yüksek duyarlılık ve özgüllükte olduğu tespit edildi. Tanıda hızlı tanı testleri güvenle kullanılabilir ancak tanının uygun olduğu durumlarda mikroskop inceleme yöntemi ve GZ-PZR yöntemleri ile desteklenmesi gerekmektedir.
Anahtar Kelimeler: Sıtma, hızlı tanı testi, gerçek zamanlı polimeraz zincir reaksiyonu, Hatay

INTRODUCTION

Malaria, which transmitted to humans through *Anopheles* spp., remains an important disease today despite the implementation of many international and national control and elimination programs (1). Increased number of travels for touristic or business purposes, especially the round trips to endemic areas ensure the continuity of malaria (2). According to the World Health Organization (WHO) estimations, there are approximately 241 million malaria cases and 627,000 deaths in 2020 worldwide. African continent was reported to host 95% of these cases and 96% deaths (1).

According to WHO data, although no autochthonous cases have been reported in Türkiye since 2010, imported malaria cases have been documented (3). The riskiest regions in Türkiye with respect to malaria are Southeastern Anatolia, Mediterranean, Marmara, and Aegean (4). The most common vector species in Türkiye are *Anofeles sacharovi* and *Anopheles superpictus* (5).

The malaria diagnosis is achieved through the detection of the parasite with microscopy in smear specimens prepared from the blood. The method called "thick drop" is the gold standard microscopic examination for malaria. However, commercially prepared rapid diagnostic tests (RDTs) and molecular methods are also utilized in the diagnosis of malaria (6,7).

RDTs, which have been developed to detect parasite-specific antigens found in the blood are one of the serological methods with supportive diagnosis that can give results in as short as 10-15 min, are easy to apply, and do not require experience (8).

Malaria RDTs contain antibodies targeting three parasitic antigens: histidine-rich protein (HRP2), Plasmodium lactate dehydrogenase (pLDH), and Pan-specific aldolase (9). HRP2 is a protein produced by only *P. falciparum* and secreted by young gametocytes and trophozoites. Hence it is used as a diagnostic marker in RDTs for detection of *P. falciparum* malaria infections (8). There are three types of pLDH used for diagnosis of one *P. falciparum*, *P. vivax*, and Pan species. This antigen shows more than 90% amino acid similarity among the four *Plasmodium* species. Since it uses monoclonal antibodies against common epitopes, pLDH-based RDT is able to detect four species. Pan-specific aldolase (Pan aldolase) is used for human-specific *P. falciparum, P. vivax, P. ovale*, and *P. malaria* (8,9). Although pLDH and aldolase are used for detection of four species, it was reported that currently available RDTs cannot specifically identify *P. malaria* and *P. ovale* (10).

The sensitivity (Se) and specificity (Sp) of the SD-Pf/Pan test were previously shown to be 99.5% and 98% for *P. falciparum* and 92.6% and 100% for non-*P. falciparum* respectively (11). As to SD-Pf/Pv test, these values were reported to be 93.9% and 98.7% for *P. falciparum* and 98.0% and 97.9% for *P. vivax* respectively (12)

The present study was planned; to draw attention to imported malaria cases in Hatay, to contribute to the treatment by subtyping the malaria cases, to determine the value of RDTs in the diagnosis of malaria cases in a short time during round trips, to analyze their practicality in the field, and to compare the performance of two different RDTs.

METHODS

Study Area and Experimental Plan

The study was carried out with the ethics committee's approval obtained from the Ethics Committee of Clinical Research at Tayfur Ata Sökmen Faculty of Medicine (approval no: 2018/86). An informed consent form was signed by all patients and documented for the current study. The patients with suspected malaria, who had round trips to malaria endemic areas between April 2019 and December 2020 and applied to the Infectious Diseases Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine, were included in the study. The gender and the history of the patients including the country they came from, were recorded. The patients were classified as positive and negative according to the findings of the microscopic examinations.

Inclusion criteria: Travelling to malaria endemic area, having clinical symptoms of malaria such as chills and fever.

Exclusion criteria: Age <18 year-old, pregnancy, lack of signed informed consent, no history of travel to malaria endemic region.

Blood samples from all patients were taken into anticoagulant containing biochemical tubes via venipuncture and sent to Hatay Mustafa Kemal University, Faculty of Medicine, Parasitology Research Laboratory. The blood samples were stored in three aliquots: One for thick drop smear preparation for the microscopic examination, another for RT-PCR analysis, and the last one for RDTs.

Microscopic Examination

Thick drop smears were prepared with the blood samples. The thick drop smears were examined to assess the presence and density of parasite. Giemsa solution was prepared by diluting it with distilled water at 1:1 ratio. Thick drop smears were stained for 45 min. Then it was evaluated with a 100X objective under a light microscope. It was considered positive when there was at least one asexual blood-stage of the parasite. By counting the number of parasites per 200 white blood cells and assuming that each subject had 8000 white blood cells per microliter of blood, parasitaemia was calculated as described previously (13). All smears were read by blinded two experienced scientists.

SD-Pf/Pan and SD-Pf/Pv Test Procedures

Two different rapid diagnosis tests were studied for all patients included in the study. One of them is SD-Pf/Pan (Product code 05FK60, Standard Diagnostics, Inc; Suwon City, Republic of Korea) (*P. falciparum*: histidine-rich protein 2 (HRP2 which can distinguish between *P. falciparum* and other types of *Plasmodium*; the other kit is SD-Pf/Pv (Product code 05FK80, Standard Diagnostics, Inc; Suwon City, Republic of Korea) which can distinguish between *P. falciparum* and *P. vivax*. The tests are in cassette format and based on the immunochromatographic antigen detection principle. Both kits were studied according to the manufacturer's protocol, and the data were recorded.

DNA Isolation and Real-time Polymerase Chain Reaction (RT-PCR)

DNA extraction was performed from the blood samples of the patients following the instructions of a commercial kit (Qiamp DNA Isolation kit, Qiagen, Germany). The obtained DNAs were stored at -20 °C until further experiments.

The isolated DNAs were genotyped by targeting the *18S ribosomal RNA* gene using a commercial species-specific qualitative RT-PCR *Plasmodium* species kit (Genesig® Std Real-time PCR detection kit for *P. falciparum, P. vivax, P. ovale, P. malariae*; Primerdesign™ Ltd., Chandler's Ford, UK) (14). RT-PCR was applied according to manufacturer's protocol. Briefly, PCR mixture was prepared for each sample and *Plasmodium* species as follow: 10 µL of oasig lyophilised 2X qPCR Master Mix (Genesig® Primerdesign™ Ltd., Chandler's Ford, UK), 1 µL of *P. falciparum* primer/probe mix, 4 µL of nuclease free water, 5 µL of DNA. The RT-PCR reaction conditions are as follow (Qiagen Rotor-Gene): Enzyme activation at 95 °C for 2 min, 50 cycles of; denaturation at 95 °C for 10 s, annelaing at 60 °C for 60 s (14). One positive control template corresponding to one malaria species was used for each PCR reaction supplied in the kit. Nuclease free water was used as the negative control. Amplification curves were assessed as positive or negative according to the amplification curves of target samples and positive controls. The most recent data were evaluated. All samples PCR reactions were run separately for each species.

Statistical Analysis

In this study, the data were analyzed with the SPPS 21 package statistical software. The data were expressed as frequency and/or percentage. Se, Sp, accuracy, and positive predictive value (PPV) and negative predictive value (NPV) were used to determine the diagnostic measure of the kits. In addition, the associations between the gold standard tests were analyzed with Mc Nemar test. The Phi coefficient measures the correlation between two nominal variables and ranges from -1 to 1. In addition, the Kappa value of the consistency of both tests (microscopy and RDT) was calculated. The formula K= (Observed consistency-expected consistency) / (1-Expected consistency) was used in the calculation. A Kappa value of 0.75 and above was considered to indicate excellent consistency.

Se, Sp, PPV, and NPV were calculated by using microscopy results as a reference, which is a gold standard method for malaria diagnosis, and by comparing with RT-PCR results at the species level.

RESULTS

Of the 66 suspected malaria patients with a travelling history to abroad (Saudi Arabia and Africa continent such as Sudan, Ivory coast, Nigeria, Uganda, Ghana), 1 was female (1.52%), and 65 (98.48%) were male. Thirty (45.45%) of the patients were positively diagnosed with microscopy. All of the patients included in the current study had a round trip history to the countries in Africa continent. Twenty-seven patients out of 30 positive patients were positive in both RDTs. In the SD-Pf/Pv test, 21 patients were positive for *P. falciparum* and 6 were positive for *P. vivax*. In the SD-Pf/Pan test, 21 cases for *P. falciparum* and 15 cases for Pan were positive (Table 1). RT-PCR was used to confirm all 30 positive patients analyzed with microscopy. Of which, 21 patients were found to be positive for *P. falciparum*, 6 for *P. vivax*, 1 for *P. ovale*, 1 for *P. malaria*, and 1 for *P. falciparum/P. malaria* as a mixed species infection (Table 2, Figure 1).

While 6 of 15 patients who were positive with SD-Pf/Pan were infected with *P. vivax* and 1 patient was infected with mixed-species of *P. falciparum/P. malaria*, 8 samples gave false positive Pan-band. In addition, 3 samples (a *P. falciparum*, a *P. ovale*, a *P. malaria*), which were positive with both microscopy and RT-PCR, were found to have false-negative bands in the SD-Pf/Pan kit while it was only one sample (*P. falciparum*) for the SD-Pf/Pv kit.

After statistical analyses, it was determined. there was a significant difference among the results obtained with microscopic examinations, both RDTs (SD-Pf/Pan and SD-Pf/Pv), and RT-PCR ($p<0.001$). While significant difference was found between SD-Pf/Pv and RT-PCR methods ($p<0.001$), there was no significant difference between SD-Pf/Pan and RT-PCR ($p>0.05$).

When microscopic results were used as the reference, SD-Pf/Pan ve SD-Pf/Pv RDTs exhibited 90% Se of, 100% Sp, 100% PPV, and 92.86% NPV (Table 3). When RT-PCR results were used as the reference, both RDTs for P. falciparum showed 95.65% Se, 100% Sp and PPV, and 88.89% NPV. Furthermore, although the SD-Pf/Pv for *P. vivax* had a 100% Se, Sp, PPV, and NPV, the SD-Pf/Pan for PAN had 77.78% Se, 61.90% Sp, 46.67% PPV, and 86.67% NPV (Table 4).

While 40.9% of the cases were diagnosed positively with the RDT test, 45.5% of the cases were diagnosed positively with microscopy. The difference was not statistically significant ($p>0.05$) (Table 5). Kappa value was calculated as 0.901.

Table 1. Comparison of microscopic examination results with the SD-Pf/Pan and SD-Pf/Pv tests in diagnosis of Plasmodium species

		SD-Pf/Pan				SD-Pf/Pv			
		Control band only	**Pf band only**	**Pan band only**	**Pf and Pan band only**	**Control band only**	**Pf band only**	**Pv band only**	**Pf and Pv band only**
Microscopy results (n=30)	Negative samples	39 (59.1%)	0	0	0	39 (59.1%)	0	0	0
	Positive samples	27 (40.9%)	12	6	9	27 (40.9%)	21	6 (22.2%)	0
	Total	66 (100.0%)	12 (44.4%)	6 (22.2%)	9 (33.3%)	66 (100.0%)	21 (77.8%)	6 (22.2%)	0 (0%)

SD: Standard deviation

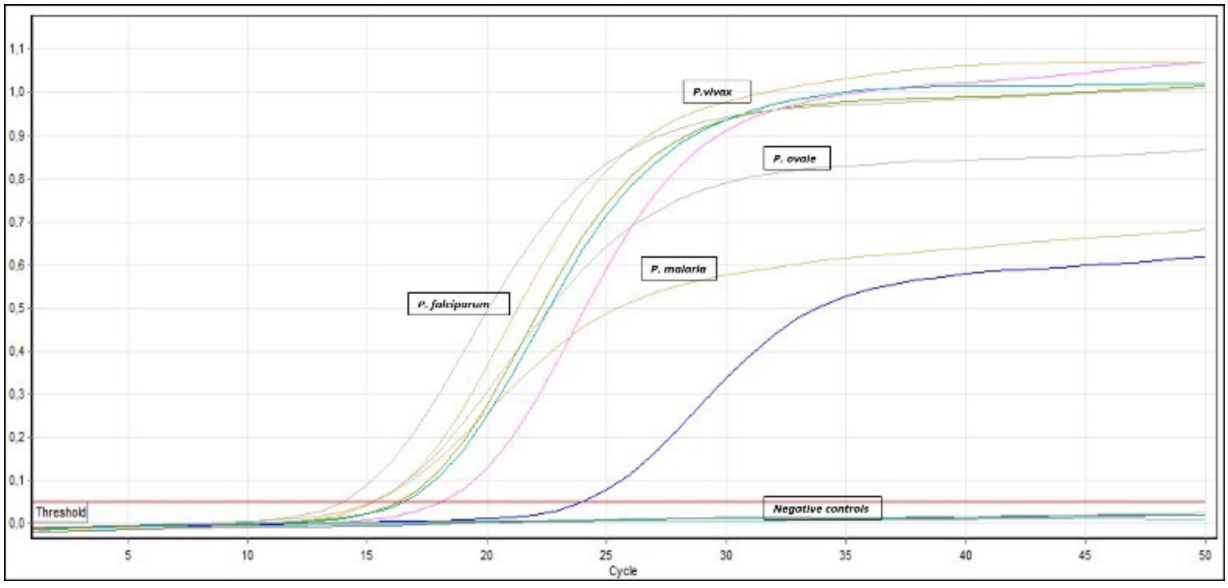

Figure 1. A representative RT-PCR amplification curve including positive control and negative controls and patient samples
RT-PCR: Real time-polymerase chain reaction

Table 2. Results obtained with microscopic examination, two rapid diagnostic tests, and RT-PCR methods

	Microscopy	SD-Pf/Pan		SD-Pf/Pv		RT-PCR				
		P.f.	Pan	*P.f*	*P.y*	*P. falciparum*	*P. vivax*	P. ovale	*P. malariae*	Mixed species infection**
Positive samples	30 (45.5%)	21 (31.8%)	15* (22.7%)	21 (31.8%)	6 (9.1%)	21 (31.8%)	6 (9.1%)	1 (1.5%)	1 (1.5%)	1 (1.5%)
Negative samples	36 (54.5%)	45 (68.2%)	51 (77.3%)	45 (68.2%)	60 (90.9%)	45 (68.2%)	60 (90.9%)	65 (98.5%)	65 (98.5%)	65 (98.5%)
Total	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)	66 (100.0%)

(15*: 15=6+1+8; 6 samples *P. vivax*, 1 sample *P. falciparum/P. malaria* mix infection, 8 samples false positives; **P. *falciparum/P. malaria* mix infection), SD: Standard deviation

Table 3. Sensitivity, specificity, positive and negative predictive values of RDTs based on the reference method of microscopy results

Reference method (microscopy)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
SD-Pf/Pan	90	100	100	92.86
SD-Pf/Pv	90	100	100	92.86

RDTs: Prepared rapid diagnostic tests

Table 4. Sensitivity, specificity, positive and negative predictive values of RDTs based on the reference method of RT-PCR results

	Reference method (RT-PCR)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
SD-Pf/Pan	P.f	95.65	100	100	88.89
	Pan	77.78	61.90	46.67	86.67
SD-Pf/Pv	P.f	95.65	100	100	88.89
	P.v	100	100	100	100

RDTs: Prepared rapid diagnostic tests, RT-PCR: Real time-polymerase chain reaction, SD: Standard deviation

DISCUSSION

According to WHO's data for the last three years, there is an increase in the rate of malaria and malaria-caused deaths each year. It has been reported that almost half of the world's population was at risk of malaria with about 14 million more cases and 69,000 more deaths in 2020 than in 2019. In the same reports, there were estimated numbers of 229 million cases and 409,000 deaths in 2019 and 228 million cases, and 405,000 deaths in 2018 (1,15).

A significant portion of malaria cases (95%) in 2020 were brought about by the countries in Africa continent and the most common malaria type was *P. falciparum*. In many countries other than sub-Saharan Africa, *P. vivax* is the primary agent in malaria infections (1).

While malaria has not been documented in Türkiye as of 2010, 81 imported malaria cases have been reported in the same year (16). Based on a report in 2019, while 75% of imported malaria cases in Türkiye originated from the African countries where the causative species was *P. falciparum*, 20% of them were caused by other Middle-East countries such as Iran, Pakistan, and Afghanistan where *P. vivax* was the causative agent. Besides among some of these malaria cases, there were mixed infections caused by multiple malaria species (17).

When we look at the imported cases reported from various provinces of Türkiye, it can be realized that the majority of these people travelled abroad for either business, e.g., drivers who transport to endemic regions, or for touristic visits. In previous studies that the following area-specific malaria cases have been reported; the 219 cases seen in Mardin in 2012 were imported cases caused by a truck drivers; between 2005 and 2015 in Gaziantep, 31 imported malaria cases; between 2012-2017 in Antalya based on Provincial Health Directorate reports 36 imported malaria cases caused by *P. vivax, P. ovale, P. malaria*, and *P. falciparum*; 22 imported malaria cases in Ankara who made round trips to various countries such as Sudan, Uganda, Cameroon, Nigeria, and Mali between 2010 and 2018 (18-21).

Imported malaria cases coming from endemic regions were also filed in Hatay province. In 2007, 4 workers coming from abroad to Hatay were infected by *P. falciparum* (22). In another study, 75 imported malaria cases who had been in sub-Saharan Africa countries in 2008-2017 were followed up and treated (23). In a study conducted with 30 imported malaria cases, who had been in malaria-endemic countries, nested-PCR method was utilized. And they found that *P. falciparum* in 16, *P. vivax* in 6, and *P. falciparum/P. vivax* mixed species in 8 cases were detected (6). In our study, 66 patients with suspected malaria, who have been in Saudi Arabia and Africa countries there and back such as Sudan, Ivory coast, Nigeria, Uganda, Ghana between April 2019 and December 2020, were included. Of the patients, 30 were found to be positive with both microscopy and RT-PCR methods, and 27 were diagnosed as positive with both RDTs. With RT-PCR, 21 cases were detected as infected by *P. falciparum*, 6 by *P. vivax*, 1 by *P. ovale*, 1 by *P. falciparum/P. malaria* mixed species, and 1 by *P. malaria* infections.

Microscopy is a highly sensitive, inexpensive, and gold standard method in malaria diagnosis providing that experienced staff and convenient equipment are available (24). Although it varies depending on the species of infectious agent, geographical location, the Se of the microscopy in malaria diagnosis is estimated as more than 75% (25). In recent years, RDTs for diagnosis of malaria, which are easy to apply and interpret, do not require advanced experience, and give results in as early as 10-15 min, have been developed and used commonly in endemic countries (8). There are types of RDTs which can detect either a single species or multiple species of malaria by targeting malaria-specific antigens such as HRP-2, pLDH, and pan-specific aldolase. WHO recommends using RDTs before starting treatment in patients with suspected malaria especially in areas where there is no required equipment and experienced staff (26). According to WHO standards published in 2011, RDTs should give 10% fewer false positives results (27). Molecular biological methods, e.g., RT-PCR, are important tools that help accurate diagnosis even at low parasitemia loads such as 5 parasites/μL and provide differential diagnosis by casting out irrelevant malaria species. Despite their high Se and Sp rates, diagnostic utility of molecular biological techniques is restricted due to the necessity of costly technical equipment (8).

In a study conducted in the Rakhine state of Myanmar for a period of 14 months, 1248 of 2585 blood samples (531 *P. falciparum*, 587 *P. vivax*, 130 *P. fal/pan* mixed species infection) were found to be positive with microscopy. They reported that 13 samples with *P. falciparum* (2.5%) gave Pan positive with RDT, 15 (2.5%) of non-*P. falciparum* samples gave false positive results as just *P. fal* or both *P. falciparum* and Pan, and 12 non-*P. falciparum* samples

Table 5. Diagnostic consistency of RDT and microscopy

			Microscopy		Total	p*
			Negative	Positive		
RDT	Negative	Count	36	3	39	0.250
		% within RDT	92.3%	7.7%	100.0%	
		% within microscopy	100.0%	10.0%	59.1%	
	Positive	Count	0	27	27	
		% within RDT	0.0%	100.0%	100.0%	
		% within microscopy	0.0%	90.0%	40.9%	
Total % within RDT % within microscopy		Count	36	30	66	
		54.5%	45.5%	100.0%		
		100.0%	100.0%	100.0%		

*Mc Nemar test, Observed Consistency =0.95; Expected consistency =0.49; Kappa value =0.901, RDT: Prepared rapid diagnostic test

(2%) gave positive bands together with *P. falciparum* and Pan. In addition, it was reported in the same study that the results of 122 samples out of 130 mixed-species infections corresponded with microscopic findings; 2 samples within the remaining 8 were Pan positive, and the last 6 samples were *P. falciparum* positive. The same study group concluded that based on the SD-Pf/Pan test results, while the Se and specificity for *P. falciparum* were 90.2% and 98.5% respectively, for Pan infection, the Se was 79.4% and the Sp was 98.7% (28). In India, 135 (20%) of 677 patients with fever were found to be positive with RDT (SD-Pf/Pan) and 86 (12.5%) with microscopy. In the same study, RDT's Se was 98.8%, specificity 91.5%, PPV 63%, and NPV 99.8%, and particularly the Se and specificity of RDT in detecting *P. falciparum* were 100% and 97.3% respectively. Moreover, they suggested that RDTs are acceptable in diagnosis of malaria as an alternative to microscopy (29). A study conducted in Africa stated that 53.8% of 437 patients were positive for *P. falciparum* caused malaria. It was also reported in the same study that while 57.8% of the samples were positive with SD-Pf/Pv, this ratio was 58% for SDPf/Pan. Although they found 92.3% compatibility with both RDTs when comparing the results of positive cases, among the negative results confirmed with microscopy, they detected 17.8% of cases as positive with SD-Pf/Pv and 18.8% cases positive with SD-Pf/Pan (30). In our present study conducted in Hatay province, a total of 30 cases out of 66 suspected malaria cases were detected as positive with microscopy and RT-PCR. Twenty-seven patients were found positive with both SD-Pf/Pan and SD-Pf/Pv tests. In the SD-Pf/Pv test, 21 patients were positive for *P. falciparum* and 6 were positive for *P. vivax*. In the SD-Pf/Pan test, 21 cases for *P. falciparum* and 15 cases for Pan were positive. With RT-PCR, 21 patients were found to be positive for *P. falciparum*, 6 for *P. vivax*, 1 for *P. ovale*, 1 for *P. malaria*, and 1 for *P. falciparum/P. malaria* as a mixed species infection.

CONCLUSION

Malaria continues to be a fatal disease. Together with the increase in the travel frequency, malaria can also be seen in non-endemic countries due to imported cases. Although Türkiye is one of the countries where malaria is eradicated, the imported malaria cases still have been documented. In the present study, the performance of RDTs was analyzed to be able diagnose the imported malaria cases in Hatay with the highest accuracy. As a result, we suggest that RDTs can be used safely in malaria diagnosis based on the microscopic examinations owing to their availability at lower costs with fewer required technical equipment compared to PCR method. Further studies with higher number of patients will help to obtain clearer and more significant outcomes.

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*** Ethics**

Ethics Committee Approval: The study was carried out with the ethics committee's approval obtained from the Ethics Committee of Clinical Research at Tayfur Ata Sökmen Faculty of Medicine (approval no: 2018/86).

Informed Consent: An informed consent form was signed by all patients and documented for the current study.

*** Authorship Contributions**

Concept: G.Ç., T.K., B.K., Design: G.Ç., Y.Ö., M.Ç., T.K., Data Collection or Processing: Y.Ö., M.Ç., G.Ç., T.K., B.K., Analysis or Interpretation: G.Ç., T.K., B.K., Literature Search: G.Ç., T.K., Writing: G.Ç., T.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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REFERENCES

1. World Health Organization. World Malaria Report; 2021. WHO: Geneva, Switzerland, Available from: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021
2. Tartar AS, Akbulut A. Fırat Epidemiological, Clinical, and Laboratory Evaluation of *Plasmodium falciparum* Malaria Cases Followed in Firat University Hospital: A 6-Year Retrospective Analysis. Turkiye Parazitol Derg 2018; 42: 1-5.
3. World Health Organization. World Malaria Report; WHO: Geneva, Switzerland, 2020; Available online: https://www.who.int/ publications/i/item/9789240015791.
4. Akdur R. Sıtma Eğitim notları. Ankara 1997. TC Sağlık Bakanlığı, Sağlık Projesi Genel Koordinatörlüğüi Sıtma Savaş Dairesi. Erişim adresi: http://www.netdata.com/UserFilesCenter3/613819aa-abf5-4ca0-b1556ba4ecdbb262/ProjectsCenter1/e903343c-0648-4473-9e7a-b0a55f9db737/FileCenter/302.pdf
5. Alten B, Çaglar SS, Ozer N. Malaria and its vectors in Turkey. Mosquito Bulletin 2000; 7: 27-33.
6. Çulha G, Zeyrek F Y, Önlen Y, Doni NY. Determination of Imported Malaria Cases in Hatay by the Use of Molecular Methods. Mikrobiyol Bul 2018; 52: 206-13.
7. Zorbozan O, Pullukçu H, Şahar EA, Karakavuk M, Hüseyin CAN, Tunalı V, et al. The Importance of the Contribution of Rapid Test, Serological and Molecular Methods in the Diagnosis of Two Imported Malaria Cases with Atypical Microscopy. Mikrobiyol Bul 2017; 51: 396-403.
8. Ardıç N, Koru Ö. Sıtmada Hızlı Tanı Testleri. Nobel Med 2012; 8: 10-5.
9. Mehlotra RK, Howes RE, Cramer EY, Tedrow RE, Rakotomanga TA, Ramboarina S, et al. *Plasmodium falciparum* Parasitemia and Band Sensitivity of the SD Bioline Malaria Ag P.f/Pan Rapid Diagnostic Test in Madagascar. Am J Trop Med Hyg 2019; 100: 1196-201.
10. Cunningham J, Gatton M, Kolaczinski K. Malaria rapid diagnostic test performance: Results of WHO product testing of malaria RDTs: round 7 (2015-2016). World Health Organization, Switzerland 2017.
11. Tadesse E, Workalemahu B, Shimelis T. Diagnostic performance evaluation of the sd bioline malaria antigen ag pf/pan test (05fk60) in a malaria endemic area of southern Ethiopia. Rev Inst Med Trop Sao Paulo 2016; 58: 59.
12. Herrera S, Vallejo AF, Quintero JP, Arévalo-Herrera M, Cancino M, Ferro S. Field evaluation of an automated RDT reader and data management device for Plasmodium falciparum/Plasmodium vivax malaria in endemic areas of Colombia. Malar J 2014; 13: 87.
13. Mfuh KO, Achonduh-Atijegbe OA, Bekindaka ON, Esemu LF, Mbakop CD, Gandhi K, et al. A comparison of thick-film microscopy, rapid diagnostic test, and polymerase chain reaction for accurate diagnosis of Plasmodium falciparum malaria. Malar J 2019; 18: 73.
14. Abamecha A, Yilma D, Addisu W, El-Abid H, Ibenthal A, Noedl H, et al. Therapeutic efficacy of artemether-lumefantrine in the treatment of

uncomplicated Plasmodium falciparum malaria in Chewaka District, Ethiopia. Malar J 2020; 19: 240.

15. World Health Organization. World Malaria Report 2019; WHO: Geneva, Switzerland Available from: https://www.who.int/news-room/feature-stories/detail/world-malaria-report-2019.
16. World Health Organization. World Malaria Report 2017. Geneva: Available from: https://cdn.who.int/media/docs/default-source/Documents/world-malaria-report 2017.pdf?sfvrsn=8b7b573a_0
17. Republic of Turkey, Ministry of Health, General Directorate of Public Health, Department of Zoonotic and Vectorial Diseases. Malaria Case Management Guide (Sıtma Vaka Yönetim Rehberi), Ankara 2019. Available from: https://hsgm.saglik.gov.tr/depo/birimler/zoonotik-vektorel-hastaliklar-db/zoonotik-hastaliklar/4-Sitma/6-Rehbler/Stma_Vaka_Ynetim_Rehberi_2019.pdf
18. Topluoglu S, Aydın A, Taylan Ozkan A, Kapcak S. P. vivax malaria cases in Mardin province in 2012-2014. 25th Congress of Clinical Microbiology and Infectious Diseases, 25 28 April 2015, Copenhagen, Denmark.
19. Özkeklikçi A, Avcioglu F. The Epidemiology of Malaria in Gaziantep Between 2005 and 2015. Turkiye Parazitol Derg 2019; 43: 102-6.
20. Ser Ö. Evaluation of malaria cases detected in Antalya Province between 2012 and 2017. Turkiye Parazitol Derg 2020; 44: 1-6.
21. Kazancıoğlu S, Bodur H. Evaluation of Imported Malaria Cases: Experience of A Training and Research Hospital in Ankara. Value Health Sci 2022; 12: 1-5.
22. Önlen Y, Çulha G, Ocak S, Savaş L, Güllü M. Yurtdışı kökenli Plasmodium falciparum sıtması: Dört olgu sunumu. Türkiye Parazitol Derg 2007; 31: 256-9.
23. Şahin S İ, Çabalak M, Bal T, Ocak S, Önlen Y, Çulha G. Hatay ilindeki importe sıtma olgularının retrospektif analizi: 10 yıl içerisinde 75 olgu. Turkiye Parazitol Derg 2019; 43: 60-4.
24. Sümer Ş. Sıtma Tanısı. Turkiye Klinikleri J Inf Dis-Special Topics 2012; 5: 20-9.
25. Wilson ML. Laboratory diagnosis of malaria: conventional and rapid diagnostic methods. Arch Pathol Lab Med 2013; 137: 805-11.
26. World Health Organization. Malaria, 2022. WHO: Geneva, Switzerland Available from: https://www.who.int/news-room/fact-sheets/detail/malaria.
27. World Health Organization. Malaria Rapid Diagnostic Test Performance. – Results of WHO product testing of malaria RDTs: Round 3 (2010-2011) Available from: https://www.who.int/tdr/publications/documents/rdt3.pdf?ua=1
28. Kosack CS, Naing WT, Piriou E, Shanks L. Routine parallel diagnosis of malaria using microscopy and the malaria rapid diagnostic test SD 05FK60: the experience of Medecins Sans Frontieres in Myanmar. Malar J 2013; 12: 166.
29. Vyas S, Puwar B, Patel V, Bhatt G, Kulkarni S, Fancy M. Study on validity of a rapid diagnostic test kit versus light microscopy for malaria diagnosis in Ahmedabad city, India. East Mediterr Health J 2014; 20: 236-41.
30. Djallé D, Gody JC, Moyen JM, Tekpa G, Ipero J, Madji N, et al. Performance of Paracheck™-Pf, SD Bioline malaria Ag-Pf and SD Bioline malaria Ag-Pf/pan for diagnosis of falciparum malaria in the Central African Republic. BMC Infect Dis 2014; 14: 109.