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Original Article

Diagnosing Malaria Cases Referred to the Malaria Reference Laboratory in Tehran University of Medical Science, Iran

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Abstract

Background: The number of malaria cases is declining worldwide; however, it remains as a serious health problem. Diagnosing unusual cases is the most important issue to manage the problem. This study designed to describe the number of *falciparum* and *vivax* malaria infected patients referred to Malaria Reference Laboratory in Tehran University of Medical Science from 2000 to 2012.

Methods: A retrospective study was conducted based on the collected questionnaires from each patient referred to the laboratory. Diagnosing results and demographic information for positive cases were analyzed using SPSS software. Problematic cases were evaluated for any difficulties in diagnosis or in clinical signs. Scanning and molecular methods were performed whenever there was an atypical case referred to the laboratory. Some of the samples had various difficulties for diagnosing such as presence of fussed gametocytes and schizonts of *Plasmodium falciparum* in peripheral blood and CCHF like hemoragic disorders.

Results: *Plasmodium vivax* caused a large proportion of the cases (76.1%) in contrast with *P. falciparum* that included smaller proportion (22.8%) and the rest (1.1) belonged to mixed infection. Most of the positive cases (69.6%) were belonged to Afghani people. Men (94.6%) showed more infection than women (5.4%), moreover the most infection (44.5%) was seen at a range of 21-30 yr.

Conclusion: In the case of existing atypical issues to diagnose, it is needed to perform more precise microscopical examination beyond the current standard conditions. Sometimes molecular method is required to verify the exact agent of the disease.

Introduction

In spite of decreasing the number of malaria cases in the world it still remains as a serious disease in tropical and subtropical areas. Approximately half of population in the world is living under the risk of malaria (1, 2). According to the report of WHO released in 2013, about 207 million new cases and 627000 deaths due to malaria occurred annually in the world mostly among the children under 5 years old (3). In some parts of the world involve with the malaria elimination program, quick diagnosis and prompt treatment with suitable antimalarial drugs are the most important recommendations of WHO.

Malaria microscopy remains as a gold standard method to diagnose the disease, but in some unusual cases, examinations that are more precise are needed. On the other hand, emergence of drug resistance in *Plasmodium falciparum* to artemisinin combination therapy (ACTs) and *P. vivax* to chloroquine increases complications of diagnosis and treatment of the infections (4-7). Therefore, more attention would be hinted about diagnosis of the infections where malaria elimination has been established (8).

Iran is a malarious country with low burden of the infection that has long been involved with malaria control program (9). Due to these control measures, the number of malaria cases reduced significantly in recent years. Prevalence of malaria in the country was reported 0.21 cases in 10,000 of population in the endemic area at 2012 (10). The most reported cases in the country were from south & southeastern Iran. Although, authorities should always be vigilant about the increasing malaria in Iran. Therefore, control and prevention of the disease is crucial for proper management in Iran, which is in elimination phase. Under these conditions, it is important to identify the exact species causing diseases, especially when there is any atypical matter in the sample. Many of referred cases to the laboratory were from endemic areas in the country or imported cases from neighboring countries where malaria is endemic. The most refugees to Iran were from Afghanistan and Pakistan, the rest of them were from some African countries, India, Ecuador and Malaysia. Therefore, it is clear that in this situation, there are many different strains with specific clinical signs or microscopic features appeared in the country. These complexity may result in wrong diagnose, so parasite maintenance may become more durable in community and then transmission conditions will occur easily. Accordingly, early diagnosis and prompt treatment of malaria cases are two essential subjects (11). Due to the complexity of features to diagnose, these cases referred to the Malaria Reference Laboratory in Tehran University of Medical Science to detect the disease.

This study was aimed to classify, discuss and describe in details considering these complicated cases, also analyze the demographic status including age, gender, nationality and Plasmodia species of those malaria patients who were referred to Malaria Reference Laboratory, School of Public Health Tehran University of Medical Sciences **(**MRL-TUMS), with emphasizing on the problematic cases.

Materials and Methods

Sample collection

From April 2002 to December 2012, overall, 521 patients were referred to MRL-TUMS by provincial malaria laboratories or academic hospitals. These patients were suspected to have malaria with some various problems that interfered for proper detection of the parasites. After registering a questionnaire form was filled and demographic data of the samples were collected.

Microscopic examination

Thick and thin blood films were prepared using finger-pricked technique. The films were

stained with Giemsa according to WHO malaria microscopy guidebook (12). Some of the samples had various difficulties for diagnosing, in these cases finding agents of the infection required long time. Therefore, microscopic examination was done beyond the standard conditions and sometimes even more than 1 h for examination just one case. Moreover, in such cases molecular examinations, PCR, were used to determine the exact agent of the disease. Finally, based on the existence of any abnormalities, these tricky samples were divided into different groups. When some abnormalities were observed in the feature of parasites, a photography process was employed for the infected blood cells using an optical microscope (Olympus BX41, with Olysia software; Olympus Corporation, Tokyo, Japan) with 100/1.40 oil objective.

Molecular identification

Molecular methods were used for those samples with having some problems to diagnose. DNA extraction, primer designing and PCR amplification were conducted in the same manner that has been described in the previous published paper (13).

Results

A total of 521 samples were analyzed in this study. Microscopically examination showed that 92 (17.6%) samples were positive for malaria parasites. Most of the positive cases (76.1%) were diagnosed as *P. vivax,* while (22.8%) and (1.1%) samples showed *P. falciparum* and mixed infections respectively (Table 1).

Table 1: Frequency of referred patients and confirmed malaria cases in Malaria Reference Laboratory, Tehran University of Medical Sciences, from April 2002 to December 2012

Year	Number of suspected patients	Malaria parasite species			Number of infected patients (%)
		P. vivax n (%)	*P. falciparum* n (%)	Mix n (%)	
2002-2012	521	70 (76.1)	21(22.8)	1(1.1)	92 (100)

The age range of the positive cases was from 1 to 87 yr. The most patients (94.6%) were male with age range of 21-30 yr old (44.5%). Groups of 11-20 (28.2%) and 31-40 (16.3%) years old were ranked in second and third places. In terms of nationality, 24 (26.1%) of cases were Iranian and 64 (69.6%) and 4 (4.3%) of cases were Afghani and other nationalities respectively (Table 2). Our findings indicated that the highest rate of the disease has occurred in July- August and October-November months (Fig. 1).

Table 2: Distribution frequency of malaria cases referred to the Malaria Reference Laboratory-TUMS related on age, gender and nationality, from April 2002 to December 2012.

Nationalities	Age (yr) / Gender										
	0-10		11-20		21-30		31-40		>40		Total
	M	F	M	F	M	F	M	F	M	F	M+F n (%)
Iranian	1	0	3	0	9	2	5	1	2	1	24 (26.1)
Afghani	0	0	22	1	28	0	9	0	4	0	64 (69.6)
Other nationalities	0	0	0	0	2	0	0	0	2	0	4 (4.3)
Total	1	0	25	1	39	2	14	1	8	1	92 (100)

Detection of parasites in low-density samples with problems required long time to solve the matters. These problematic cases were categorized into two groups; the first was about those samples which had difficulties in parasite diagnosis that means existence abnormalities in parasite appearance or were clinically suspected patients that parasite may not found in the standard microscopy method. The second was about those patients with specific sign except usual symptom in malaria disease that were led to misdiagnosis by physicians. In a few cases, diagnosing process took more time than standard test and results were confirmed with molecular approaches (for two samples) as mentioned above. Cases with abnormalities in morphology of parasites such as round gametocytes instead of banana shape and schizont in peripheral blood in *P. falciparum* infection were illustrated in Fig 2, 3.

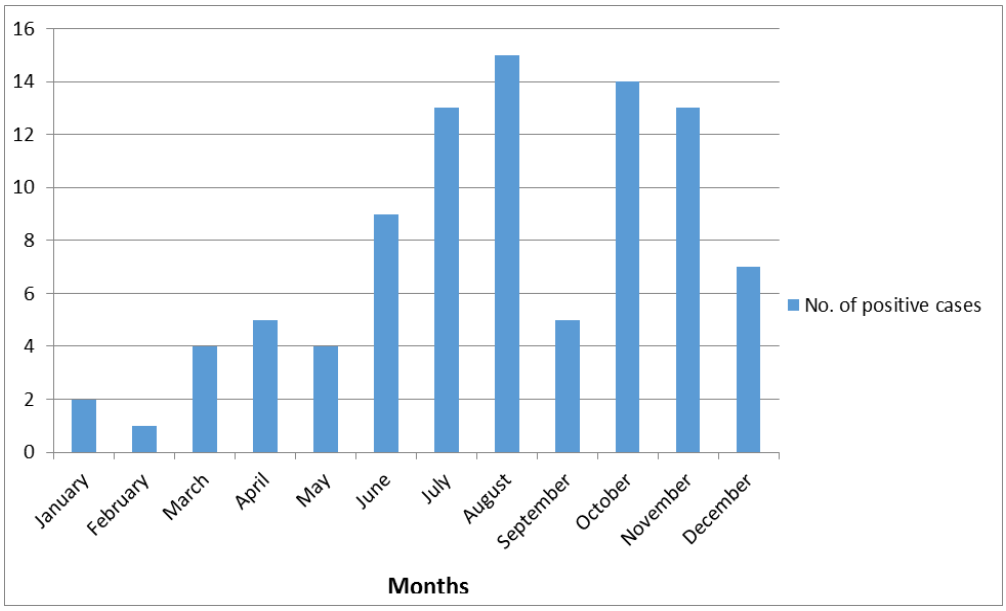

Fig. 1: Monthly distribution of malaria positive cases during 2002 to December 2012 referred to MRL-TUMS

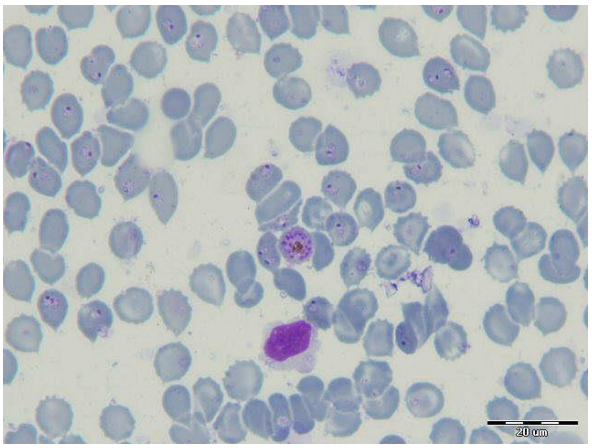

Fig. 2: A shizont in peripheral blood of a patient infected with *Plasmodium falciparum* (Original)

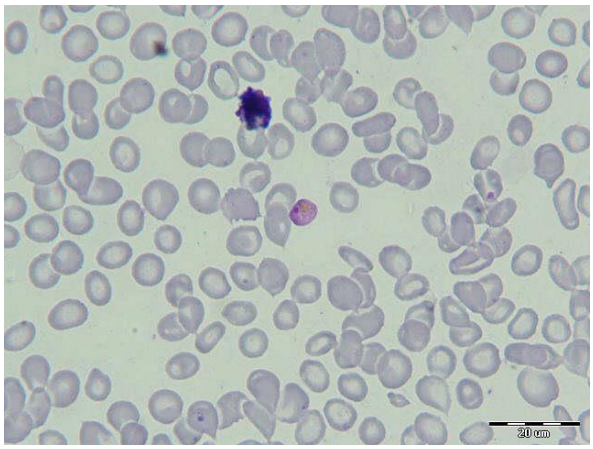

Fig. 3: A round shape of *Plasmodium falciparum* gametocyte (Original)

Discussion

Despite of performing malaria control program in Iran, the disease is still a public health problem in southeastern Iran. Imported cases from eastern neighboring countries Afghanistan and Pakistan are a major problem against the elimination of malaria in Iran. Although, Tehran as capital of Iran is not a malaria endemic area however since economic, social and political importance of the city includes thousands of different workers and travelers from the malaria endemic areas.

According to the recorded data in the TUMS Malaria Reference Laboratory, most of the positive cases were Afghani and Pakistani travelers and those Iranian travelers who came back from south Asia and African countries. More cases were observed among men than women due to more entrance of men in this country for finding a job. The results obtained in this study are in agreement with Khalili's findings who reported that 4257(95%) cases were male and 225 (5%) were female from the total of 4482 confirmed malaria positive cases obtained from Yazd province during 1985 to 2006 (2). Sargolizaie and colleagues have done an extensive study in Sistan & Baluchistan Province during 2008 to 2011, and found that 77.3% of the positive cases were Iranian and 22.7% from foreign nationalities, in contrast to our study that showed 74% and 26.1% were foreign nationalities and Iranian respectively (14). This significant difference is because our study was performed in non-endemic area, but Sargolizaie et al. conducted their study in an endemic area in Iran. Moreover, they reported that 88.6% of the cases were *P. vivax* and 11.4% *P. falciparum* (14). Prevalence of *Plasmodia* species pattern in the country more or less is the same. In the present study, most of the positive cases that were mostly from malaria endemic area, were *P. vivax* (76.1%) while 22.8% and 1.1% of the cases were *P. falciparum* and mixed infections respectively. Khalili et al. found approximately the same results as Sargolizaie and colleagues (2, 14), such results also have been reported by the other authors from different malarious areas (15-18), as well as by the Center for Disease Control in Iran (10).

Based on the demographic data of this study the number of referred cases were higher in July to August and October to November than other months, comparable to those result reported from Khorasan Province (18). The greatest number of positive cases lay on the age group of 21-30 years. It is clear that most of the infections have occurred in working age people who came from the neighboring countries to Iran. Similar results obtained by other researchers from Iran (15-18).

Some of the referred cases to the laboratory possessed numbers of diagnosing difficulties, such as low parasitemia, mixed infections and abnormalities in morphology of parasites or existence of parasites in non- standard samples (for example bone marrow), also some of them had clinically symptoms with specific signs except common malarious symptoms, like hemorrhagic fever. As it was mentioned above, these abnormalities were classified into two groups, first: complexity in parasite appearance in blood smears and second complications in symptoms of the disease.

In the case of complexity in blood smears, there were several important points that should be considered for example; mixed infections either over growth with *P. vivax* or *P. falciparum* that diagnosing process takes more time than usual standard microscopy examination. Another issue was probability of sequestration phenomenon in complicated *falciparum* malaria that may induce to hidden parasites. In this case, coma likely happens whereas parasites might not see in the peripheral blood of patients. However, it is advisable that such patient would be hospitalized due to symptoms while treatment of malaria is being considered for him/her as a supposition treatment.

Occurrence of unusual symptoms or atypical sign in a patient may lead to a miss under-

standing and diagnosing the disease by physician. One of the highly controversial cases referred to the laboratory was from an area where kala-azar disease had been common there, a slide was taken of bone marrow of a patient who suspected to kala-azar, but some *P. falciparum* rings were seen in RBCs when a microscopist was looking for *Leishman* body in the BM slide. Recovery happened when the patient was treated with administering anti-*falciparum* malaria drugs. The other case was about a patient who had severe bleeding. He was hospitalized because of suspected to Crimean–Congo Hemorrhagic Fever (CCHF), but blood smears indicated infection of *P. vivax* .He recovered after taking antimalarial drugs. The shape of parasites was normal in both of the above-mentioned cases, but atypical symptoms caused misleading by physicians.

Finding parasite in patients with low parasitemia (less than 500 parasites/ml) took more time than usual standard time, similar to mixed infections. In patients infected with *P. falciparum* observing a few number of schizonts in stained blood films may lead microscopist to misdiagnose. Obviously, we can consider the presence of *P. falciparum* shizonts in peripheral blood in some specific strains or when the patient enters in to hyper parasitemia phase (Fig. 2) (19).

Another unusual shapes that may be seen in the peripheral blood is *P. falciparum* round gametocyte (Fig. 3). Although it is expected to see banana shape gametocyte for *P. falciparum,* in some cases round shape gametocytes may confuse a professional microscopist.

Conclusion

The referred cases to MRL-TUMS mimicked those cases detected in the field in incidence, but in the case of abnormal forms that may be seen in the peripheral blood, microscopists should be trained further and have enough experience.

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The authors declare that there is no conflict of interests.

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