Original Research Article

Survey in Iranian Malaria blood donation deferral: Laboratory evaluation in Malaria blood donation deferral in the Iranian Blood centers

ABSTRACT

Introduction:

Transfusion-transmitted malaria (TTM), from a malaria infected donor, is an accidental Plasmodium infection, which is one of the concerns of blood transfusion issue in the world. Countries have adopted different strategies for donor selection and improving criteria to overcome these concerns,
In the Iranian Blood Transfusion Organization (IBTO), donors who refer to blood transfusion centers after registration are screened by the physicians based on interview and examination. This prevention due to the possibility of malaria infection is called malaria donor deferral strategy.

Methods:

In this study 248 malaria blood donation deferral samples were selected and evaluated by microscopic, serological and molecular methods, after completing the questionnaire.

Results:

The results of the survey of the questionnaires showed that 30 cases were deferred from donating blood due to having a history of malaria infection. In microscopic examination, Plasmodium falciparum ring was observed in 2 samples and 2 samples were also reported as suspicious. In serological examination, 23 cases (9.27%) were reported positive. All cases were reported negative for the presence of malaria parasite DNA.

Conclusions:

The adoption of TTM prevention strategies depends on the malaria endemicity of the geographical area. According to the IBTO instructions, donors are examined and interviewed by a blood transfusion physician's and owning to traveling or residency in endemic area or malaria infection, deferred temporary or permanently.
Recent study reveal that the strategy of donor selection and deferral in the IBTO, with little residual risk of malaria transmitting, is sufficient to prevent the occurrence of TTM in Iran.

Key words: Malaria, Blood donation deferral**,** Microscopic method, Serological method, Molecular method, Iran

Introduction

Malaria is the most serious, important and widespread human parasitic disease in the world. More than two hundred and forty million people are infected every year and about six hundred thousand people die due to this disease (1).

Numerous infectious agents are transmitted through the blood, including malaria, and the possibility of its transmission through blood products, especially red blood cells, is one of the concerns of blood transfusion authorities in the world (2).

Transfusion-transmitted malaria (TTM) by contaminated blood or blood component is an accidental Plasmodium infection and causing high-risk complications by directly releasing malaria parasites into the recipient's bloodstream. In some cases, it can be fatal, especially in people who have not been previously exposed to malaria or people with immunodeficiency (3).

If malaria transmitted through blood transfusions is not diagnosed and treated in time, it may cause the blood recipient to die (4).

While in some countries, such as Brazil with about 0.06% prevalence in eligible donors, malaria screening in blood banks is performed under a microscopic method (5), there is no standard proven laboratory method in most countries. Therefore, one of the best TTM prevention approach are performed through interviews and physical examination and deferred of suspicious donors. However, the presence of asymptomatic carriers in malaria-prone areas as well as donors who do not remember the history of infection or travel to malaria-prone geographical areas, It is still a risk factor for blood transfusions (6).

There are several factors that negatively effect on blood availability. Numerous deferral criteria are the most important of these factors. In recent years, increasing communication and demographic changes in societies, increased the number of donor deferral due to travel to malaria endemic areas (7).

To avoid TTM, donors' deferral is performed based on history of travel or residency in endemic areas or history of infection, and donors with these risk factors are temporarily or permanently deferred from blood donating according to the different blood center strategy in various countries. While the likelihood of these people being infected, especially travelers to endemic areas, is very low. For this reason, blood transfusion centers have always been thinking of alternative solutions to deal with malaria (8).

Although blood transfusions are considered a vital treatment, they are still a potential source of disease transmission. Therefore homovigilance, in addition to other blood care effects, is important in malaria-prone in terms of blood care and malaria prevention especially in low income countries (9).

In Iranian blood transfusion organization (IBTO) as headquarter of blood transfusion centers, the medical history is confirmed by conducting a medical interview and examination by an expert medical physician (10).

Donor selection or screening and deferral system, can prevent TTM in endemic and non-endemic countries (11), hence effective strategies to overcome the blood safety concern are essential for any country.

Malaria has been a serious threat in Iran in past decades due to its high mortality rate, but currently malaria endemic areas are limited to the southeastern provinces of Sistan and Baluchestan and the southern provinces of Hormozgan and Kerman (12). On the one hand blood donation are not investigated using parasitological, immunological and molecular laboratory assays in Iran. On the other hand owing to the importance of the quality and quantity of donated blood, as well as the limited studies that have been performed on the evaluation of the malaria blood donation deferral individuals; the aim of this study was to evaluate the parasitological parameters in the deferral blood donor due to the possibility of malaria.

Materials and Methods

This study has been performed by cross-sectional method. Population in this study was 248 donors that deferred from blood donation in 13 blood transfusion centers in Isfahan, Alborz, Bushehr, Tehran, North Khorasan, Razavi Khorasan, Khuzestan, Sistan and Baluchestan, Fars, Kerman, Golestan, Hormozgan and Yazd provinces. They have been deferred for a period of one or three years or permanently for various reasons, such as a history of malaria disease, traveling or residency in endemic areas. According to the Ministry of Health in 2018 and IBTO in 2019, in Hormozgan province only Jask, Bashagard and Rudan cities, and in Sistan and Baluchestan province only Nikshahr, Sarbaz, Chabahar, Konarak, Iranshahr, Mehrestan and Saravan cities and in Kerman province Only Qale-e-Ganj, Manojan, Kahnooj, Rudbar-e-Junub cities and rural areas of Jiroft city are considered as endemic areas and others are categorized as non-endemic provinces. In order to investigate presence or absence of Plasmodium parasites and antibodies, screening was performed microscopically and serologically, and then, positive or suspicious samples were examined by molecular method.
The questionnaires were completed and signed by the people themselves based on written consent and then blood samples were collected. The questionnaire included information such as gender, age, education, residency, occupation, income, blood donation history, deferral history and traveling to malaria-prone area history. Samples were examined microscopically after preparation of thin and thick peripheral blood smears and staining. Blood samples were centrifuged and tubes were stored in minus 20 ° C for serological and molecular tests.

Table 1- Malaria blood donation deferral due to infection or travel

	Province	No.	Due to infection	Due to travel
1	Esfahan	81	No	81
2	Alborz	11	No	11
3	Bushehr	11	No	11
4	Tehran	17	No	17
5	North Khorasan	26	No	26
6	Khorasan Razavi	9	No	9
7	Khuzestan	1	No	1
8	Sistan and Baluchestan	18	18	No
9	Fars	16	No	16
10	Kerman	14	No	14
11	Golestan	8	No	8
12	Hormozgan	13	11	2
13	Yazd	23	1	22
Total		**248**	**30**	**218**

Microscopic examination

From each participants, 5cc blood samples were collected and two thin and Thick Smear were prepared and stained with Giemsa dye and examined using a light microscope.

Serological examination

Serological evaluation was performed by indirect ELISA method using laboratory kit DIA.PRO/Milano-Italy Malaria Ab Lot 0717 / AG to determine specific antibodies in plasma samples. The wells in the microplate were covered with recombinant protein antigens containing the human species Plasmodium epitopes (Vivax, Malariae, Falciparum, Ovale, and Knowles), which are able to show human species Plasmodium antibodies. By adding a

plasma sample to the wells, if the sample contains any types of IgG, IgM, IgA antibodies, complex between antibodies and recombinant antigens is established. Then biotin recombinant proteins, and Hourseradish Proxidase (HRP) enzyme was added respectively. HRP acts on antigen and antibody complexes through hydrolytic activity and Peroxidase substrate led to the spread of blue color during the incubation period. The intensity of the blue color depends on the amount of Plasmodium's human antibody antibodies and determines the presence or absence of antibodies in the plasma. Finally, the stopper solution was added and the optical absorption (OD) was measured by an ELISA reader at 450 nm.
All controls including positive control, negative control and Cut Off were repeated in all tests and all samples were duplicated.

Molecular study
DNA extraction was performed using ACCU-PreP® kit, genomic DNA extraction kit (BI-ONEER, Seoul, Korea), and according to the manufacturer's instructions. PvLDH and PfLDH genes were amplified using conventional polymerase chain reaction (PCR) and primers listed in Table 2 (13).

Table 2- PvLDH and PfLDH gene amplifying primers

PvLDH gene amplifying primers	
Forward	5′-ATGACGCCGAAACCCAAAAT-3′
Reverse	5′-ACCTTTAAATGAGCGCCTTCAT-3′
PfLDH gene amplifying primers	
Forward	5′-AGATGGCACCAAAAGCAAAAAT-3′
Reverse	5′-ACCTTTAAGCTAATGCCTTCAT-3′

*Pv*LDH primers were designed based on *P. vivax* Belem (DQ060151.1) and *P. vivax* Sal-1 (XM_001615570.1) strain, While *Pf*LDH primers were designed based on *P. falciparum* 3D7 (XM_001349953.1) reference sequence, both from GenBank.
The whole blood DNA of a healthy person living in a non-endemic area were used as negative controls in amplification process. PCR reaction containing 1µL of Forward and Reverse primers (10 pmol), 10µL Mastermix (Amplicon, Denmark) containing Tris-HCl with pH 8.5, 1.5 Mmol MgCl2, dNTPs and TaqDNA polymerase, 3µL of genomic DNA samples and 10µL of distilled water in 25 µL for each Reaction.
PCR cycle parameters for PvLDH gene amplification were as follows: 5minutes initial denaturation at 95 °C followed by 30 cycles with 30 s at 95 °C, 30" at 56 °C, 1′ at 72 °C and final extension at 72 °C for 5 min. All the PCR parameters were the same for PfLDH gene amplification except the annealing temperature was 58 °C. The PCR products of PvLDH and PfLDH were loaded on 1% agarose gel. The gel contained SimplySafe (EURx, Poland) for DNA staining. UV transilluminator was used to visualize the stained DNA. The fragment sizes of PCR products were determined using 1kb DNA ladder marker (Solis BioDyne, Estonia) (13).

Results

The results of the questionnaires showed that 30 cases were deferred from blood donation due to their history of malaria infection. Of these, 18 were from Sistan and Baluchestan and 11 from Hormozgan. The last malaria blood donation deferral case belongs to Yazd, which is a

non-endemic province, but he has resided in Sistan and Baluchestan for 2 years. All 30 were aware of their malaria infection history.

Results of microscopic examination

Based on the preliminary results of microscopic examination obtained from the observation of 248 thin and thick peripheral blood smears, a total of 70 samples were reported as suspicious, of which 40 cases, they were deferred due to travel to malaria-prone areas, and 30 cases were blood donors who had a history of malaria infection in the past. Suspected thin and thick peripheral blood smears were sent to the National Malaria Diagnosis Laboratory at the School of Public Health in Tehran University of Medical Sciences for approval as a malaria reference laboratory. As a confirmed microscopic result, Plasmodium falciparum rings were observed in 2 samples and 2 samples were reported as suspicious. The characteristics of positive and suspicious samples are summarized in Table 3.

Table3- Characteristics positive and suspicious samples reported by the National Malaria Diagnosis Laboratory

Province	Sistan and Baluchestan	Sistan and Baluchestan	Hormozgan	Isfahan
Microscopic result	Positive	Positive	suspicious	suspicious
Gender	Male	Male	Male	Male
Year of birth	1976	1995	1976	1990
Residency	Fanuj county	IranShahr	Bandar Abbas	Isfahan
Infection history	yes	yes	yes	No
Travel history	No	No	No	To Bandar Abbas

Results of serological examination

All 248 malaria blood donation deferral samples were serologically analyzed using DIA.PRO/Milano ELISA kit. Based on the results 23 (9.27%) samples were positive for the presence of anti-Plasmodium antibodies. 20 positive serological samples (8.06%) related to endemic provinces (Sistan and Baluchestan, Hormozgan and Kerman) and 3 samples (1.21%) related to non-endemic provinces (Isfahan and Yazd). On the other hand, out of these 23 positive serological samples, 20 samples were related to deferred cases who had a previous history of malaria infection (11 cases from Sistan and Baluchestan, 8 cases from Hormozgan and 1 from Yazd who has lived in Sistan and Baluchestan for 2 years). 3 of these 23 samples were related to defer from blood donation for reasons such as traveling to malaria-prone or staying in these areas (2 cases from Isfahan and 1 from Kerman province). The results are summarized in Table 4.

Table 4- Results of serological examination

	Province	**positive samples**	**Infection history**	**Travel history**
1	Sistan and Baluchestan	11	11	No
2	Hormozgan	8	8	No
3	Kerman	1	No	1
4	Efsahan	2	No	2
5	Yazd	1	1	No
Total		**23**	**20**	**3**

Results of molecular study

All samples that were considered positive or suspicious, whether microscopic or serologic, were molecularly analyzed by PCR. Of these, 30 samples were related to those who had a previous history of malaria infection and the remaining 40 samples were deferred for traveling to or staying in malaria-prone areas as mentioned before. Samples were sent to the National Malaria Diagnosis Laboratory at the School of Public Health in Tehran University of Medical Sciences and were molecularly analyzed by Real Time PCR. All samples, including positive and suspicious microscopic and also positive serological samples, were negative for the presence of malaria parasite DNA.

Discussion

Blood-borne malaria (TTM) is rare in non-endemic countries, while it is a serious threat in endemic areas (14), because in addition to causing health problems and illness to the recipient, can in some cases lead to death (15).

In non-endemic countries, there are proper guidelines for donors that gather travel information as well as the potential risk of malaria. People in non-endemic countries usually do not have a symptomatic infection after traveling to malaria-prone areas. Accordingly the first approach to identifying malaria risk, is a history of travel or residence in malaria-prone areas, as well as the duration of residence and the previous history of malaria should also be considered. Each country, depending on its own circumstances and criteria, shows a different strategy in dealing with deferral system from blood donation, and the biggest difference in these strategies is the policy of testing versus not testing (16).

In Canada, for example, since July 1995, donors who have any history of malaria infection, have been permanently deferred from the blood donation cycle (17), while in the United States, donors diagnosed with malaria are deferred for three years after being asymptomatic (18). In the UK, donors diagnosed with malaria are permanently removed from the blood donation cycle in the absence of a valid malaria test, but if a valid test is possible, 4 to 6 months after complete treatment, they can return to the blood donation system (19).

In Brazil, malaria screening in blood banks relies on microscopic examination (5) and in India, which is one of the endemic areas and bears the main burden of Plasmodium falciparum and vivax, malaria is diagnosed by methods such as microscopic examination and rapid diagnostic test (RDT) (20).

Forasmuch as malaria transmitted through blood transfusions, especially in endemic areas, is a threat to blood health (16), on the other hand rapid identification of Plasmodium parasites is very important for the treatment and monitoring of patients (21), so it is essential for any country to have an effective screening method or proper strategies for donor selection and improvement of donor deferral system criteria (16). The most strategies were used are screening of blood donors through interviewing or laboratory tests (22).

However, in most countries, including Iran, there is no proven laboratory method for screening donors, and prevention of malaria transmission can only be determined through interviews and physical examination (6). Iran is one of the countries that has chosen the policy of deferral system through interviews and physical examination without testing. Iranian blood donors who travel to endemic malaria-prone areas are deferred from blood donation for 1 year and for 3 years if they reside in these areas and donors with a history of malaria will be permanently deferred from donation system. Before starting the malaria eradication program (MEP) in Iran, 344 cases of transfusion-transmitted malaria (TTM) were reported from 1963 to 1983. Although no cases of TTM have been reported until 2015 (23).

A retrospective study in Iran showed that almost 0.18% blood donation were deferred due to the risk of malaria (22).

The most widespread diagnostic method is the microscopic examination of thin and thick peripheral blood smears, also known as the standard diagnostic test. The sensitivity of this method can vary depending on the individual's expertise (24).
NAT-based methods using parasitic nucleic acid can reduce the risk of TTM. These methods are very effective in identifying infected volunteer donors and there is a good prospect for routine screening to prevent TTM (5).
Prevention of transfusion malaria depends on the elimination of infected donors (15), however none of the usual methods are sensitive enough to be used in blood banks (25).
Compared to traditional malaria diagnostic methods such as microscopy and rapid testing (RDT), DNA-based methods, are highly sensitive and provide a certain diagnosis of Plasmodium species and mixed infections. DNA-based methods, however, has not yet been optimized as a routine, but extensive use of PCR can help fill in the gaps obtained by traditional methods (21).
Total number of studies performed by microscopic diagnostic method on malaria cases caused by blood transfusion in non-endemic areas, was reviewed and compared in a systematic review study in 2018. Plasmodium species were reported with a frequency of 45% falciparum, 30% malaria, 16% vivax, 4% ovale, 2% nolsia and 1% mixed infection of falciparum and malaria (3).
In another study, with the aim of investigating the presence or absence of Plasmodium and then accurately identifying the parasite species in donors in Kampala and Ginja regions of Uganda, 1000 blood bags were examined using Nested PCR. Malaria parasitic infection was reported among asymptomatic blood donors in these two regions as 15.4% and 87.7%, respectively (26).
Also, in a study in Iran, which was conducted from 2005 to 2010 to investigate the deferral malaria process in Tehran province, 2,827,129 blood donation volunteers were examined, of which 23,462 had been deferred due to probability of malaria infection. Statistical analysis showed that the highest percentage of malaria deferral (99%) was due to travel to endemic areas (27).
In sub-Saharan Africa, the prevalence of malaria in non-malaria-infected blood recipients was examined over a period of one year. The prevalence of malaria due to blood transfusion in patients receiving Plasmodium falciparum infected blood was 4.7% under microscopic examination, 13.7% in rapid diagnostic test, 18% in PCR and 22.2% in enzyme immunoassay (EIA) (25).
In another study in Ghana, the prevalence of Plasmodium parasitic infection in blood donors by rapid malaria diagnostic test (RDT) and microscopy was 8% and 3%, respectively, while in non-donors it was 5% and 2%, respectively (28).
In a recent study, in the microscopic examination, 2 positive and 2 suspicious samples were reported. In serological examination by ELISA, 9.27% samples were positive for the presence of anti-Plasmodium antibodies. All samples, were negative for the presence of malaria parasite DNA. According to the low number of positive cases no significant relationship between the questionnaire information was observed.

Conclusion

According to the World Malaria Global Strategy and the National Strategic Plan for Malaria Elimination in 2010, it is anticipated that by 2025, Iran will be declared a malaria-free zone (1). Iran has seen a significant reduction in malaria incidence in recent decades and has been recognized as a progressing elimination region since 2009 (29). Also, based on the World Health Organization report in 2021, no native cases of malaria have been reported in Iran for three consecutive years, and the incidence and mortality rate has shown a significant decrease (1).

It is important for blood transfusion organizations that adopt strategies to malaria identification due to prevent potential TTM. The adoption of TTM prevention strategies depends on the malaria endemicity of the geographical area.
Iran is one of the countries that has chosen the policy of not screening for malaria and in fact has chosen deferral system, since malaria is endemic in the few parts of Iran (Cities in Sistan and Baluchistan, Hormozgan and Kerman provinces). In IBTO, donors are interviewed and examined by an expert transfusion physician before donation. According to the written instructions, donors who have been infected with malaria during their lifetime are permanently, and donors who have traveled to or resided in malaria-prone areas, are temporarily deferred.
Another noteworthy point is that Iran has had a significant increase in malaria control in terms of endemicity in recent years, according to WHO statistics. On the other hand, the results of a recent study emphasize the importance of interviewing and examining by an expert transfusion physician, and it seems that currently the donor deferral system is the ideal method and there is little residual risk of TTM.
the strategy of donor selection and deferral in the Iranian Blood Transfusion Organization is sufficient to prevent the occurrence of transfusion-transmitted malaria (TTM) in Iran. It should be mentioned that the adoption of TTM prevention strategies depends on the malaria endemicity of the geographical area. Since malaria is endemic in the few parts of Iran (Cities in Sistan and Baluchistan, Hormozgan and Kerman provinces), the strategy to prevent TTM in these provinces should be different from non-endemic areas, while this is not the case.

Availability of data and material

All data and materials are available.

Consent to participate

In the consent form, the consent for participation is stated.

Consent for publication

In the consent form, the consent for publication is indicated.

Ethical approval

The questionnaires were completed and signed by the people themselves based on written consent. All procedure performed in the study involving human participants, were in accordance with the ethical standards of national research committee Number: IR.TMI.RED.1397.015 and IR.TMI.RED.1395.012

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