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Action plan to regain unnecessary deferred blood donors due to malaria risk in Turkey

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ABSTRACT

Malaria was expected to be a major problem during blood donation in Turkey due to existence of malaria cases in southeastern region of Turkey. The present study aimed for the first time, to investigate malaria in “donors deferred for malaria risk” and to determine the regional rates of malaria deferral in Turkey. Blood samples were collected from several Blood Banks of southeastern provinces where local malaria cases still exist and from Blood Bank of Ege University Medical School (EUMS) located in western Turkey where malaria is eradicated decades ago. *Plasmodium* spp. and specific antibodies were investigated by stained smears, antigen detection, PCR and ELISA. Among the donors deferred for malaria risk, *Plasmodium* spp. were not detected by microscopy, PCR or antigen detection. Seroprevalances were 2% and 3.92% in western and southeastern regions, respectively. Rate of donor deferral for malaria risk was 0.9% in EUMS and deferrals were exclusively because of travel to southeastern Turkey. In southeastern provinces, deferrals were mainly due to malaria like fever history. The present study first time assessed regional rates of donor deferral due to malaria risk in Turkey. Previously, malaria was expected to be a major problem during blood donation in Turkey due to existence of malaria cases in southeastern region of Turkey. The results of the study showed that 97% of the deferrals were unnecessary. In conclusion, to reduce unnecessary donor deferrals in Turkey, in addition to comprehensive questioning for malaria history, the usage of a malaria antibody screening method should be initiated prior to deferral decision.

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1. Introduction

Plasmodium spp. can easily be transmitted through cellular blood products and may cause lethal malaria in the recipient especially if the species is *Plasmodium falciparum* [1–3]. After the first report of transfusion transmitted malaria in 1911, increment of travel freedom, economic status and migration movements have possessed

an increased risk to blood recipients [4–6]. According to the global review of Bruce-Chwatt, about 350 transfusion transmitted malaria cases were reported from 1911 through 1950 and 1,756 cases between 1950 and 1972 [7]. The incidence of transfusion transmitted malaria in United States was <0.1 cases per 10⁶ transfusions between 1990 and 2005 [8]. In Australia, approximately 1 million donations are collected annually and the estimated transfusion transmitted malaria is reported to be less than one in 15 million since 1991 [3]. In England, five cases of transfusion transmitted malaria have been reported from 1986 through

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2004 [4,9]. In France, 120–150 cases were detected between 1960 and 1989 and decreased to six cases during the period of 1990–2006 [2].

The decrease of transfusion transmitted malaria cases in non-endemic countries was mostly due to collection of comprehensive medical and travel history from donors prior to donation [2,3,9,10,11]. Although the intense questioning of donors for malaria was effective, it resulted in many unnecessary donor rejections and in Ireland, rejections were permanent [3,5,6,8,11]. In Australia, the estimated loss of red blood cells per year reached approximately 35,000 donations or 5% of annual donation [3]. In United States, among 535,211 donations, 2.9% of donations were deferred due to malaria in 1998, and from 2000 through 2006, among 29 million donors 1.1% were deferred [6,8]. According to another evaluation, the amount of deferral was more than 100,000 donations per annum in United States [10,11]. Most of the time, unnecessary deferral disheartens the donor and many of them never return to give blood [11]. Nowadays, serological screening tests detecting malaria antibodies are being developed to reduce the rate of deferrals [1,5,12–16].

Currently, the blood product demand is over 1 million donations per year in Turkey due to increasing number of transplantation surgeries, improved intensive supportive treatment strategies for cancer or infectious diseases. According to world malaria report local cases of malaria still exists in Turkey. In 2008, reported cases decreased to 136 of which 49 were imported. Most of the local cases belong to southeastern provinces of Turkey and malaria transmission is exclusively due to *Plasmodium vivax* [17]. In Turkey, donors are mainly screened for malaria risk by “donor questionnaire” form and a malaria screening assays is not routinely in use. Although the donors can easily be rejected due to malaria risk, the rate of malaria deferral, the presence of *Plasmodium* spp. and malaria seroprevalence in deferred donors were not investigated in Turkey. Although, almost 60 transfusion transmitted malaria cases have been reported in Turkey previously, a recent transfusion transmitted malaria case has not been reported in the last 5 years [18–21].

A study about the presence of *Plasmodium* spp. in “donors deferred for malaria risk” has not been conducted frequently in literature [22]. In Turkey, the presence of *Plasmodium* spp. was only investigated in the eligible donors [23–25]. Thus, the present study aimed to investigate first time the presence of *Plasmodium* spp. and *Plasmodium* specific antibodies in “donors deferred due to malaria like fever or travel to malaria endemic regions” in order to determine the regional rates of malaria deferral and regional malaria seroprevalence rate in “donors deferred for malaria risk” in Turkey. The data that will be acquired from the present study is expected to help assess the future action plan for the malaria screening strategy in blood donors in Turkey.

2. Materials and methods

2.1. Deferral guidelines

In Turkey, donors are mainly screened for malaria risk by “donor questionnaire” form prepared in accordance with

the instructions of National Blood and Blood Components Guideline of Republic of Turkey, Ministry of Health that was published in 2009. According to the guideline, donors who has malaria history, traveled to malaria endemic regions in the past 6 months, and malaria like fever history without any other illness should be deferred, unless malaria specific antibodies are not detected [25]. In the guideline, the deferral criteria of donors for malaria risk are as follows:

1. *Donors that lived in malaria endemic region in the first 5 years of life:* These donors are deferred for 3 years after their last visit, unless malaria symptoms are not detected. This period can be reduced to 4 months if the result of an immunologic or molecular assay is negative.
2. *Donors that had malaria attack:* These donors are deferred for 3 years after their treatment and symptoms related to malaria have ceased. They can be eligible for donation if the result of an immunologic or molecular assay is negative.
3. *Asymptomatic donors that have traveled to endemic regions:* These donors are deferred for 6 months after they moved to non-endemic region unless the result of an immunologic or molecular assay is negative.
4. *Donors that experienced undiagnosed fever during or after 6 months of their visit to endemic regions:* These donors are deferred for 6 months after their symptoms have resolved. This period can be reduced to 4 months if the result of an immunologic or molecular assay is negative.

2.2. Subjects studied, donors and inclusion criteria

Since malaria mainly exists in southeastern provinces of Turkey (Fig. 1), almost all of the major Blood Banks in this region (listed below) were included to the study [17].

1. Dicle University Medical School (DUMS) Blood Bank located at Diyarbakır. DUMS is the biggest university hospital in the region and serves mostly to people living in southeastern Turkey.
2. Turkish Red Crescent, Southern Anatolia District (TRC–SAD) Blood Service collecting donations from Diyarbakır, Şanlıurfa, Batman, Siirt, and Mardin where almost all of the malaria cases have been detected in 2008.



Fig. 1. Map of Turkey showing İzmir, Diyarbakır and Şanlıurfa provinces (Black colored) where the study is conducted. Gray colored areas are Mardin, Siirt, and Batman provinces where local malaria cases also exist [17].

3. Harran University Medical School (HUMS) Blood Bank located at Şanlıurfa. HUMS has smaller capacity compared to DUMS and serves mostly to people living in southeastern Turkey.

In addition, Blood Bank of Ege University Medical School (EUMS), located in western region of Turkey where local malaria cases do not exist for decades, was included to the study (Fig. 1) [17]. EUMS is the biggest university hospital in western region of Turkey and mostly serves to people living in western region of Turkey.

Blood samples were collected from donors deferred for malaria risk that admitted to above mentioned Blood Banks, between April 2008 and 2010. Initially, all donors filled out “Donor Questionnaire” form prepared according to the National Blood and Blood Components Guideline [25]. Then, all donors were questioned about their medical and travel history by experienced doctors/medical staff. The first donor deferral criteria of guideline was not applied during questioning of donors in Blood Banks of southeastern Turkey since almost all of the questioned donors were born and living in southeastern Turkey. In addition, third donor deferral criteria was partly applied during questioning of donors in Blood Banks of southeastern Turkey (donors that lived in southeastern Turkey were only deferred due to travel to malaria endemic countries outside Turkey).

Deferred donors due to malaria risk were recruited into five distinct groups as follows: (1) donors that lived in southeastern region of Turkey in the first 5 years of life where local cases of malaria still exists (2) donors that lived in elsewhere malarious area in the first 5 years of life (3) asymptomatic donors that have traveled to southeastern Turkey (4) donors that have traveled to malaria endemic countries (5) donors deferred due to malaria like fever history.

Control group is composed of eligible healthy donors selected randomly from the EUMS Blood Bank (n : 50) and DUMS Blood Bank (n : 50). All volunteer donors were provided with written informed consent prior to collection of blood samples as approved by the Ege University Medical School, Research Ethics Committee.

2.3. Giemsa staining

Thin and thick blood smears were prepared from the venous blood collected in tubes containing EDTA and stained by Giemsa (Merck, Germany) as described and examined for the presence of *Plasmodium* spp. [26]. Giemsa stained slides were examined under light microscopy with immersion oil approximately for 15 min by two qualified parasitologists.

2.4. OptiMAL Rapid Malaria test

OptiMAL Rapid Malaria test (DiaMed, Switzerland) detects and differentiates *Plasmodium* spp. by the presence of *Plasmodium* spp. lactate dehydrogenase (pLDH) using monoclonal antibodies directed against isoforms of pLDH. OptiMAL was used according to the manufacturer’s protocol. Positive and negative controls provided by the kit were used.

2.5. Pan malaria antibody CELISA

Anti-*Plasmodium* antibodies were analyzed using pan malaria antibody CELISA according to the manufacturer’s protocol (Cellabs, Australia). Serum samples diluted 1:100 were incubated with microwells of the plate were coated with a panel of recombinant malaria proteins. After probing wells with conjugate provided by the kit, bound antibodies were visualized after adding chromogen substrate. Thereafter reaction was stopped and optical density (OD) values were evaluated in a micro titer plate reader (Bio-Tek EL×808) at 450 nm. The cutoff value for each run was determined to be the mean OD of two negative controls (provided by the kit) plus 0.100. Reactive serum samples were retested in duplicate and repeatedly reactive sera were considered seropositive.

2.6. DNA extraction and nested PCR analysis

Isolation of DNA from donors’ venous blood samples was performed by using High Pure PCR Template Preparation kit according to the manufacturer’s protocol (Roche, Germany). Nested PCR amplification reactions targeting 18s subunit ribosomal gene of *P. falciparum* (GeneBank No.: AF145334), *P. vivax* (GeneBank No.: AF145335), *P. malariae* (GeneBank No.: AF145336), and *P. ovale* (GeneBank No.: AF145337) were performed as previously described [27]. The first 50 µl final volume reaction included 2.5 µl purified template DNA, 0.25 µM genus specific primers (rPLU6 and rPLU5) (Table 1), 1 U of Taq Polymerase (Stratagene, USA), 1× reaction buffer, 200 µM dNTPs (Invitrogene, USA), 2 mM MgCl₂. The initial PCR amplification reaction was performed with the following calculated protocol: 5 min initial denaturation step at 95 °C, followed by 25 cycles of 1 min at 94 °C, 2 min at 58 °C, and 2 min at 72 °C, and a final extension of 5 min at 72 °C. The second 50 µl final volume reaction included 2.5 µl of the first PCR reaction product, 0.25 µM of each species specific primers (Table 1), 1 U of Taq Polymerase (Stratagene, USA), 1× reaction buffer, 200 µM dNTPs (Invitrogene, USA), 2 mM MgCl₂. Second amplification reaction was similar to the first reaction, except 30 cycles were performed.

Positive control plasmids containing species specific 18s subunit ribosomal gene fragment (*P. falciparum*: MRA-156; *P. vivax*: MRA-178; *P. malariae*: MRA-179; *P. ovale*: MRA-180) were obtained from MR4-ATCC (Malaria Research and Reference Reagent Resource Center-American Type Culture Collection, Manassas, VA, USA). In each reaction, two positive controls containing 10 and 1 copies of species specific 18s subunit ribosomal gene fragment/µl sample, were used. Distilled water was used as negative control.

2.7. Statistical analyses

Data obtained during the study were processed using Prism 3.03 (GraphPad, San Diego, CA). A two-tailed unpaired *t* test with 95% confidence interval was used to determine the significance between the results of assays.

Table 1
Genus and species specific primers used to detect *Plasmodium* spp. by nested PCR [27].

| | Primer | Sequence | Product size |
|----------------------|--------|--|-----------------------|
| Genus specific | rPLU6 | 5'-TTAAAATTGTTGCAGTTAAAACG-3' (23 nt, forward primer) | Approximately 1200 bp |
| | rPLU5 | 5'-GAAGTTTAAGGCAACAACAAG-3' (21 nt, reverse primer) | |
| <i>P. falciparum</i> | rFAL1 | 5'-TTAAACTGGTTGGGAAAACCAATATATT-3' (30 nt, forward primer) | 206 bp |
| | rFAL2 | 5'-GACGGGTAGTCATGATTGAGTTCATTGTGT-3' (30 nt, reverse primer) | |
| <i>P. vivax</i> | rVIV1 | 5'-CGCTTCTAGCTTAATCCACATAACTGATAC-3' (30 nt, forward primer) | 121 bp |
| | rVIV2 | 5'-TAAGGACTTTCTTTCCTCGGCTTGGAAAGT-3' (30 nt, reverse primer) | |
| <i>P. malariae</i> | rMAL1 | 5'-ATAACAAAGTGTACGTTAAGAATAAACGC-3' (30 nt, forward primer) | 145 bp |
| | rMAL2 | 5'-TTTGTATAATTTTTATGCATGGGAATTTT-3' (30 nt, reverse primer) | |
| <i>P. ovale</i> | rOVA1 | 5'-ATCTCTTTTGTCTATTTTTAGTATTGGAGA-3' (30 nt, forward primer) | 788 bp |
| | rOVA2 | 5'-CACTAGGATACAATTAATGTCTCTTT TCC-3' (30 nt, reverse primer) | |

3. Results

3.1. Eligibility of donors and deferrals due to malaria risk in western region

During the study, 41,195 donors have admitted to Blood Bank of EUMS and 30,170 were found eligible for donation. Among 11,025 non-eligible donors 87 of them (0.79%) were deferred because of travel to southeastern provinces of Turkey. In addition, 13 donors (0.11%) were deferred because of travel to malaria endemic countries. Overall rate of donors deferred at malaria risk was 0.9% (Table 2.). Among the deferred donors, 44% of them reside in western Turkey.

3.2. Eligibility of donors and deferrals due to malaria risk in southeastern region

In the same period, 32,794 donors have admitted to TRC–SAD Blood Service and 26,779 were found eligible

for donation. None of the donors were deferred due to malaria risk among the 6,015 non-eligible donors (Table 2.).

Among the donors who admitted to DUMS, which was the other main Blood Bank of the region, 42,934 were found eligible and 89 donors were deferred due to malaria like fever history and 13 donors were rejected because of travel to malaria endemic countries during the study. All of the deferred donors reside in southeastern Turkey. Data about the amount of donors admitted to Blood Bank and non-eligible donors were not available (Table 2).

In addition, among the 13,254 donors who were found eligible at HUMS Blood Bank, none of the donors were deferred due to malaria like fever history. Data about the amount of donors admitted to Blood Bank, non-eligible donors and donors deferred due to travel to malaria endemic countries were not available (Table 2).

None of the donors were deferred due to living in elsewhere malarious areas worldwide in the first 5 years of life.

Table 2
Donors deferred due to malaria like fever history and travel to malaria endemic regions between April 2008 and 2010.

| Name of the Blood Bank | Number of donors admitted to Blood Bank | Number of donors found eligible for donation | Number of donors found non-eligible for donation | Number of the donors deferred because of travel to southeastern provinces of Turkey ^a | | Number of donors deferred because of travel to malaria endemic countries | Number of the donors deferred due to malaria like fever history |
|--|---|--|--|--|---|--|---|
| | | | | Donors that lived in southeastern Turkey in the first 5 years of life | Asymptomatic donors that have traveled to southeastern Turkey | | |
| İzmir Ege University Medical School | 41,195 | 30,170 | 11,025 | 56 | 31 | 13 | 0 |
| Diyarbakır Dicle University Medical School | NA | 42,934 | NA | 0 | 0 | 13 | 89 |
| Şanlıurfa Harran University Medical School | NA | 13,254 | NA | NA | NA | NA | 0 |
| Turkish Red Crescent, Southern Anatolia District Blood Service | 32,794 | 26,779 | 6015 | 0 | 0 | 0 | 0 |

NA: data not available.

^a Diyarbakır, Şanlıurfa, Mardin, Siirt, and Batman are southeastern provinces of Turkey where local malaria cases still exist [17].

3.3. Giemsa stained thin and thick smears, OptiMAL Rapid Malaria Test, and nested PCR

Plasmodium spp. were investigated in blood samples of 202 non-eligible donors deferred for malaria risk and 100 control group donors eligible for donation. *Plasmodium* spp. were not observed during the microscopic examination of stained thin and thick smears. In addition, *Plasmodium* lactate dehydrogenase (pLDH) enzyme produced by all forms of the parasite was not detected. Furthermore, 18S subunit ribosomal gene of *Plasmodium* spp. was not detected by nested PCR (data not shown). Nested PCR detected *P. vivax*, *P. falciparum*, *P. malariae*, and *P. ovale* specific 18S subunit ribosomal gene fragments in positive controls. Amplified plasmodial DNA was not observed in negative controls.

3.4. Antibody results in deferred donors' serum samples

Among 202 donors deferred for malaria risk, six samples were seropositive (2.97%). In the control group, none of the donors were seropositive. The overall malaria seroprevalence in donors deferred for malaria risk in southeastern provinces of Turkey was 3.92% (4/102) and slightly higher than EUMS Blood Bank located in western region where the seroprevalence was 2% (2/100) ($P = 0.42$).

4. Discussion

Transfusion transmitted malaria occurs rare in malaria non-endemic countries however it is a leading cause of potential blood product wastage. Moreover, unnecessary deferral due to malaria risk disheartens the donor and many of them never return to give blood [1–3,5,6,8,11,16]. Current research has focused on regaining the disheartened donors, decreasing wastage of potential blood products by discussing on questionnaire forms and developing novel cost effective screening assays for malaria.

Previously, in most malaria non-endemic countries, individuals who have lived in the first 5 years of their life in malaria endemic areas were rejected for 3 years after their last visit to the endemic area. In France, the donors were deferred for 4 months if the immuno fluorescent antibody (IFA) test specific for malaria was negative. In Ireland, such donors were permanently rejected. Donors from most non-endemic countries who visited malaria endemic regions were accepted for donation 6 months after their return with exception of France accepting for donation after 4 months if the IFA test was negative. Ireland has stringent regulations resulting in the permanent deferral of the donor who stayed at malaria endemic regions for more than 6 months [2,6,16,28].

In Australia, according to the Australian Red Cross Blood Service Guidelines, donors that visit malaria endemic country were restricted to only plasma donation for 12 months. Donors who stayed in malaria endemic region for 6 months or more within the last 3 years and donors with history of malaria were restricted to plasma donation for 3 years. This strategy minimized transfusion transmit-

ted malaria cases however wastage of red blood cells increased to 5% of annual donation [3]. In United States or Canada, donors who have traveled to malaria endemic regions deferred for 1 year after their return and those who are residents of an endemic country were deferred for 3 years after departure from that country. This strategy caused a significant increase in donor deferral and it is estimated that more than 540.000 donations were lost between 2000 and 2006 [6]. According to a study, the deferral rate of donors for malaria risk in several European countries range between 0.003% and 0.43% of all donations [28].

Turkey was a malaria endemic country at beginning of the century however a national malaria elimination strategy and relevant plan of action have been prepared to eliminate the disease by 2015 [17]. In Turkey, donors are mainly screened for malaria risk by "donor questionnaire" form and a malaria antibody screening assay is not routinely in use. In addition, the rate of malaria deferral, the presence of *Plasmodium* spp. and malaria seroprevalence in deferred donors were not investigated in Turkey.

Thus, several questions have aroused to be addressed. What is the malaria deferral rate especially in southeastern Turkey where local malaria cases exist? Is comprehensive questioning still enough or is inclusion of a malaria antibody screening assay is requisite as the deferral guideline suggests? What must be the future action plan to regain unnecessary deferred blood donors due to malaria risk in Turkey?

The accumulated data obtained from the questionnaire form of the deferred donors showed that, in western Turkey rate of donor deferral due to malaria risk was found to be 0.9% among non-eligible donors and 0.24% among all donors. Deferrals were exclusively because of travel to southeastern provinces of Turkey or infrequently because of travel to endemic countries. In southeastern provinces, a rate of donor deferral was not assessed due to the lack of data about donors admitted to Blood Bank and non-eligible donors. According to the data obtained from deferred donors, deferrals were mainly due to malaria like fever history and rarely because of travel to malaria endemic countries. DUMS and TRC-SAD Blood Service are two main blood product providers in southeastern Turkey. Interestingly, none of the donors were deferred due to malaria risk by TRC-SAD Blood Service and deferrals were mainly due to malaria like fever history in Blood Bank of DUMS (Table 2).

In Turkey, *Plasmodium* spp. were almost always investigated in eligible blood donors who applied for donation in Turkey [22–24]. In Şanlıurfa (located in southeastern Turkey), *Plasmodium* spp. were not detected in 5000 donors who applied for donation using stained smears [23]. In another study, *Plasmodium* spp. were investigated using stained smears and OptiMAL in 2229 donors who applied to Blood Banks in Istanbul (located in western Turkey) and in Adana (located in southeastern Turkey) [22]. In a recent study, *Plasmodium* spp. were not detected among the 1850 donors who applied for donation using stained smears and OptiMAL in Blood Bank of DUMS [24]. In the present study, the presence of *Plasmodium* spp. or anti-*Plasmodium* antibodies were investigated in blood samples of 202 non-eligible "malaria at risk" donors using micro-

scopic examination of stained slides, antigen detection, PCR and ELISA.

Microscopic examination of stained smears is the most widely applied diagnostic test for malaria however it is time consuming and the sensitivity of Giemsa stained thick and thin smears decreases markedly at samples containing 5–20 and 50–200 parasites/ μl , respectively [9,29–31]. Malarial antigen detecting assays are rapid and objective compared to microscopy but on the other hand, lower sensitivity than microscopy impedes their routine use. Comparison of microscopy with OptiMAL (detection threshold is 100–200 parasites/ μl of blood) in several studies showed that showed that OptiMAL has a sensitivity of 91.3–98% and a specificity of 92–100% [9,30–33]. The most sensitive PCR method can detect as low as 0.004 parasites/ μl of blood [9,31]. In the present study, a nested PCR described by Snounou et al. was used to detect the presence of *Plasmodium* spp. in blood samples of deferred donors [26,34–36]. In a study comparing blood smear, OptiMAL and nested PCR, the sensitivity and specificity of microscopy and OptiMAL were 50–29.1% and 100–95.6%, respectively, using nested PCR as gold standard [34]. The same nested PCR protocol and microscopy were used to assess malaria presence in 129 samples. The results showed that *Plasmodium* spp. were detected in 46 samples (35.6%) by nested PCR and in 37 samples (28.7%) by microscopy [35].

Currently, serological screening tests detecting anti-*Plasmodium* antibodies are being developed to reduce the rate of deferrals and assess a malaria seroprevalence for donors in non-endemic countries [1,5,12–16]. Although IFAT is the reference test, ELISA is more sensitive, objective and suitable for screening donors. [1,12,13,29]. In two studies conducted in United Kingdom, seroprevalence rates in donors at risk of malaria were 1.5% (with ELISA detecting *P. falciparum*) and 5.47% (by ELISA detecting *P. falciparum* and *P. vivax*) [12,13]. Seroprevalence rates were 1.33% (10/751) in Australia and 1.7% in New Zealand [13,39].

More recently, molecular tools were used to determine the presence of *Plasmodium* spp. in seropositive “malaria at risk donors”. *Plasmodium* spp. were investigated in blood samples of seropositive donors in Australia (2697/135.225; 1.99%) using antigen detection and PCR. Only one of the seropositive donors was positive with PCR. As the population was increased to 250.000 donors, two of them were PCR positive [3,40]. In a study conducted in France, malaria seroprevalence was 4.2% (454/10.615) in malaria at risk donors, using IFAT and ELISA. PCR was negative among the 98 of IFAT and ELISA discordant samples [5]. Afterwards, 3.5% of the blood donations were tested for anti-*Plasmodium* antibodies and 0.89% of them were positive by ELISA in France [2]. Similar to the present study, a semi nested PCR was used to investigate the presence of *Plasmodium* spp. in 125 deferred blood donors in Spain. Among them, PCR detected five malaria cases (4%) whereas microscopy was negative. Authors suggested that, PCR can serve as reference test for donors at malaria risk to increase blood donations and shorten deferral period [21].

In the present study, antibodies against all four forms of the malaria were investigated in “donors deferred for malaria risk” using CELISA. Studies using consensus result

(obtained by Giemsa staining, IFA, or ELISA methods) as gold standard showed that, the sensitivity and specificity of CELISA were reported as 83–95.5% and 85–92.2%, respectively [37,38]. In the present study, malaria seroprevalence in “donors deferred for malaria risk” in western and southeastern regions of Turkey were 2% and 3.92%, respectively. *Plasmodium* spp. were not detected using microscopy, antigen detection and PCR. However it must be kept in mind that molecular methods can detect parasitemia in a small portion of blood product and malaria can still be transmitted through transfusion owing to the large size of blood products that may contain extremely low levels of parasitemia (i.e. <0.004 parasites/ μl of blood) [9]. The absence of *Plasmodium* spp. in blood samples of deferred donors using microscopy, OptiMAL and PCR in the present study, do not essentially suggest the nonexistence of *Plasmodium* spp. since donors might be asymptomatic carriers with low parasitemia [9]. Overall, 97% of deferrals monitored in the present study were definitely unnecessary since anti-*Plasmodium* antibodies were not detected in serum samples. In addition, these results showed that antibody screening in combination with questioning can be an effective approach to reduce unnecessary donor deferral due to malaria risk in Turkey. However, presence of malaria specific antibody is not an indication of parasitemia and an additional assay detecting parasitemia may be required to further prevent the wastage of potential blood products [1,3,5,9,12,13,28,30,42].

The National Blood and Blood Components Guideline used in the present study was published in 2009 and in 2011, a new guideline was published [25,41]. In the new guideline, donors that lived in malaria endemic region in the first 5 years of life are no longer rejected. In the present study, 56 donors (56%) from western region of Turkey were rejected because of this criteria since previous guideline was used during the study. According to the results of serology, none of them had malaria risk. Therefore, all of the deferrals due above criteria were definitely unnecessary supporting the removal of the above criteria from the 2009 guideline.

The present study first time assessed a regional rate of donor deferral due to malaria risk in a western province where local malaria cases do not exist for decades. Deferrals due to malaria risk were not observed in TRD–SAD Blood Service that accepts donation from southeastern provinces of Turkey where local malaria cases still exist. Although deferral data about donors in the remaining two Blood Banks that serve to southeastern region are partially missing, an idea formed about the deferral causes in that particular region. In addition, anti-*Plasmodium* antibody screening first time assessed regional seroprevalence rates about the “donors deferred for malaria risk” in Turkey. Therefore, in the action plan of Turkey, donor questionnaire form must be updated frequently to decrease unnecessary deferrals due to malaria risk since malaria is almost eliminated in Turkey. In addition, the results of the study show the necessity of using antibody screening tests in combination with questioning to reduce unnecessary donor deferral as well as requirement of PCR to further prevent the wastage of blood products in Turkey.

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