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## Brief Report

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# DETECTION OF *PLASMODIUM* PARASITES IN HEALTHY BLOOD DONORS USING POLYMERASE CHAIN REACTION

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Malaria is an important tropical disease in terms of morbidity and mortality. It is difficult to identify hidden contaminated cases through the use of ordinary techniques, such as microscopic methods, in endemic regions. In the present study, two techniques were used to assess the rate of contingent malaria contamination in healthy inhabitants: microscopic study (thin and thick blood smears) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). One hundred blood samples were collected from healthy individuals who lived in Chabahar, southeastern Iran, in June 2002, during peak mosquito season.

Three positive samples (3%) were detected by PCR method, all of which had been reported negative by microscopic examination. It seems that the most appropriate method for population-based screening of blood donors for malaria parasites could be through the detection of parasite nucleic acids using PCR. Such a strategy would also reduce transfusion-transmitted malaria in endemic areas.

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**Keywords:** Blood donors • Iran • malaria • PCR-RFLP • transfusion malaria

### Introduction

Malaria is one of the important problems in many countries, especially in tropical areas.<sup>1</sup> Infection with malaria parasites may cause a variety of clinical symptoms, depending on a combination of different factors, including the virulence of the parasite isolate, host-related factors, such as the immune status, and genetic make up.<sup>2, 3</sup> The symptoms of disease are nonspecific and include headache and pains in the back and limbs, anorexia, nausea, chill, and continuous or remittent fever.<sup>4</sup> Anemia and frequent malaria relapses affect the labor force and,

in turn, the economic and agricultural status of a society.

Today, more than 90 countries are located in regions with endemic malaria; more than half of these are African countries. The South-East of Iran is a malaria-endemic area.<sup>5</sup> The nonsexual form of malaria has been detected in the peripheral blood of patients. Many reports from southeastern Iran have detected chloroquine-resistant *Plasmodium falciparum*.<sup>6, 7</sup> This can be diagnosed randomly or through special tests studying other infections.

Infected blood donors are considered a potential hazard for blood recipients. The transmission of malaria via blood transfusion is the second manner of transmission (in which the sexual cycle and tissue phases are omitted) and is associated with acute manifestations. Using direct smear, Kinde-Gazard et al<sup>8</sup> showed that 33.5% of the donors carried various species of the parasite but had no symptoms. In this study, *P. falciparum* had the highest incidence (99.6%). Unfortunately, due to the complex and delayed detection of parasites in donors in endemic regions, blood recipients are at serious risk of transmission-related malaria

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infection.<sup>9</sup> To combat this, medical centers must have a role in providing health services and safe blood in endemic regions. This descriptive and cross-sectional study is an analysis of the presence of the malaria parasite in 100 healthy blood donors in endemic areas of Iran.

## Materials and Methods

### Samples

One hundred healthy blood samples were randomly selected from the blood bank in Chabahar, Sistan and Baluchestan Province in southeastern Iran.

### Microscopic screening

To detect the malaria parasites, at least 100 microscopic fields of thin and thick blood smears were studied with immersion lens (X100 magnification) for each sample.

### Primers

The sequences of 18S rRNA gene of four human *Plasmodium* parasites (*P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*, all taken from the gene bank [www.ncbi.nlm.nih.gov/nucleotide](http://www.ncbi.nlm.nih.gov/nucleotide)) were compared by DNAsis software, and two pairs of primers of their common pieces were designed for nested polymerase chain reaction (PCR).

PCR I forward and reverse primers were: Plas1F 5'- AAC CTG GTT GAA CTT GCC AGT AGT -3' and Plas1R 5'- CCA AGC TAC TCC TAT TAA TCG TAA C-3'. Nested PCR forward and reverse primers were: Plas2F 5'- TAT ATG TAG AAA CTG CGA ACG GTC -3' and Plas2R 5'- CAA CTA CGA GCG TTT TAA CTG CAA -3'.

### DNA extraction

The DNA extraction from blood samples was carried out using Sambrook and Russell's method.<sup>10</sup>

### PCR reaction

The PCR reaction contained 3 microliters of extracted DNA (approximately 50 ng), 2.5 microliters of 10X PCR buffer, 0.1 mM dNTP, 20 picomol each of forward and reverse primers, 1 unit of Taq DNA polymerase and up to 25 microliters of deionized water. A 900-bp fragment of 18S rRNA gene was amplified. Nested PCR reaction was done with 2 microliters from PCR product as a template. Hence, a 564-bp fragment of

that gene was amplified. The cycling parameters were as follows: initial predenaturation at 94°C for 5 minutes, 30 cycles consisting of denaturing at 94°C for 30 seconds, annealing at 64°C for 30 seconds (external and internal primers), and 72°C for 30 seconds and the final extension at 72°C for 5 minutes.<sup>10</sup>

### Agarose gel electrophoresis

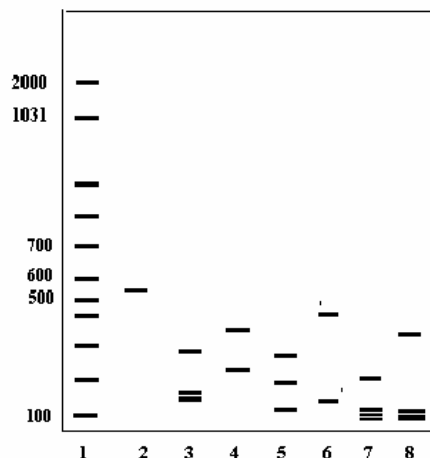
The PCR product was electrophoresed on 1.5% agarose gel, stained by ethidium bromide and DNA bands were visualized at 254 nm by UV transilluminator.

### RFLP

The specific PCR products of the *Plasmodium* species were determined using restriction enzymes. According to the schematic pattern (Figure 1), *HinFI* could differentiate *P. falciparum* and *P. malariae* from *P. vivax* and *P. ovale*. Further discrimination of species is carried out via *HeaIII* and *Tsp45I* that differentiate *P. falciparum* from *P. malariae* and *P. vivax* from *P. ovale*, respectively.

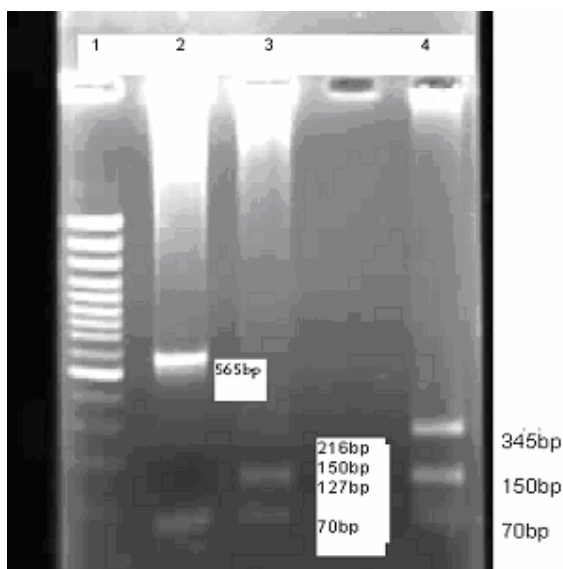
## Results

Using the PCR method, 3 samples (3%) were



**Figure 1.** Schematic pattern of human *Plasmodium* species differentiation by PCR – RFLP.

Lane 1: 100 bp DNA ladder marker; Lane 2: PCR product; Lane 3: pattern of *P. vivax* and *P. ovale* after digestion by *HinFI*; Lane 4: pattern of *P. falciparum* and *P. malariae* after digestion by *HinFI*; Lane 5: pattern of *P. vivax* after digestion by *Tsp45I*; Lane 6: pattern of *P. ovale* after digestion by *Tsp45I*; Lane 7: pattern of *P. falciparum* after digestion by *HeaIII*; and Lane 8: pattern of *P. malariae* after digestion by *HeaIII*.



**Figure 2.** Agarose gel electrophoresis. Lane 1: 100 bp DNA ladder marker; Lane 2: PCR product; Lane 3: *P. falciparum* pattern (HaeIII); and Lane 4: *P. malariae* pattern (HaeIII).

detected positive for the 18S rRNA gene of *Plasmodium* parasites, which were reported negative by microscopic screening. As shown in Figure 2, when PCR product was affected by the *HinFI* restriction enzyme, the pattern of *P. falciparum* and *P. malariae* were observed on agarose gel. Then, PCR product was affected by *HeaIII* restriction enzyme and the pattern of *P. malariae* was observed.

## Discussion

The transmission of malaria by blood transfusion is a serious risk, as the diagnosis of malaria in the recipient is unexpected, and this is often missed.<sup>8</sup> Microscopic examination of donor blood is highly unsatisfactory as most infected donors infections are evident only at a submicroscopic level.<sup>11</sup> Outside of endemic areas, the policy of screening the donor's history for known episodes of clinical malaria or for tropical travel in the past 5 years is generally sufficient.<sup>4</sup> In view of the increasing frequency of tropical travel in the general population, the policy in some countries is to reject only donors whose malaria serology is positive.<sup>4</sup> This screening method is not perfect,<sup>11</sup> but a negative serology gives a high probability of freedom from infection. The use of PCR<sup>12</sup> as a highly sensitive method for malaria is widely recommended.<sup>13-17</sup>

This research focused on the possibility of

parasite existence in healthy individuals. Infected persons may act as a distribution agent of malaria, with further transmission through mosquito bites. Asymptomatic persons may also be considered healthy donors.<sup>18</sup> The aim of screening tests is to isolate those who have been exposed to the infectious agent, so the test should have repeatable criteria, reasonable costs, high accuracy, and sensitivity.

Results of Kinde-Gazard's, et al study<sup>8</sup> suggested that in spite of drug-resistant malaria, more systematic care must be directed toward blood screening. Many researchers have considered different sequences as the target gene, for example, 18S rRNA gene for nested-PCR by Snounou et al,<sup>19</sup> the 18S rRNA gene for RT-PCR by Abdullah et al,<sup>1</sup> and mitochondrial DNA by Tan et al.<sup>20</sup> The choice of target DNA is important for the sensitivity of the method. There are 1000 to 10,000 copies of the 18S rRNA gene in eukaryotes, while this value in human *Plasmodium* is reported to be about 10 copies.<sup>21</sup>

Because of high sensitivity of the PCR technique and its importance for malaria detection in transfusion, Mungai et al<sup>22</sup> have suggested the use of this technique along with a screening interview. Today, donated blood units are screened through serological methods (IFA) and interviews in Europe, where malaria is not prevalent. In these countries, the low contamination rate causes the rejection of blood units in doubt. But, following such a strategy leads to the wasting of blood products in endemic area. There are many reports<sup>23-25</sup> that show asymptomatic malaria-infected individuals. There are some reports of transfusion-transmitted malaria.<sup>22, 26-30</sup> Blood transmitted *P. malariae* has been reported in Russia,<sup>31</sup> China,<sup>32</sup> Turkey,<sup>33</sup> Congo,<sup>34</sup> Iran,<sup>35</sup> and Canada.<sup>30</sup>

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