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**Original Article**

# High-Level Expression of Immunogenic Recombinant *Plasmodium vivax* Merozoite Surface Protein (*Pvmsp-1* $_{42}$ kDa) in pGEX 6P1 Vector

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**Abstract**

**Background:** Detection of *Plasmodium vivax* specific antibodies with serological tests could be a valuable tool for epidemiological researches. Whereas *P. vivax* cannot be simply obtained in vitro, serological tests using total or semi-purified antigens are infrequently used. Given this restriction, the present study investigated whether recombinant *P. vivax* merozoite surface protein 1 (PvMSP-1 $_{42}$ kDa) could be useful in detection of antibodies from the serums of a *P. vivax* infected person using serological tests.

**Methods:** Parasite DNA was extracted from blood sample of an Iranian *P. vivax*-infected patient. The region of PvMSP-1$_{42}$ kDa was amplified by PCR then cloned into pTZ57R/T vector and sequenced. The insert was sub cloned into pGEX 6P1 expression vector. Afterwards, it was transformed into *E. coli* BL21 and cultured massively. Sub cloning of gene was confirmed by PCR and enzyme digestion and sequencing finally. Production of recombinant protein was confirmed by SDS-PAGE. Western blot was performed by human sera to appraisal binding ability to the IgG antibodies of *P. vivax* infected patients. Recombinant protein was purified and estimated by Bradford assay.

**Results:** The specialty values of the Western blot determined with 10 sera from naturally infected individuals, 10 sera from healthy individuals and 7 sera from individuals with other infectious diseases.

**Conclusion:** For the Iranian population, using a Western blot assay for MSP-1$_{42}$ recombinant protein can be used as the foundation for promotion of serological assay for the detection of *P. vivax* malaria such as ELISA.

**Keywords:** *Plasmodium vivax*, Recombinant *PvMSP-1* $_{42}$ kDa, Expression vector, Iran

## Introduction

*Plasmodium vivax* (*P. vivax*) is the second most frequent human malaria parasite. It has been estimated that 2.85 billion people all around the world are at risk of this infection (1). Compared to the lethal counterpart, *P. falciparum,* which a few months to few years after the initial blood-stage infection, the infection could be treated, *P. vivax* could be relapsed and expanded. It may also be cases of chloroquine resistance or severe complications. In spite of focused efforts to reduce the mortality rate caused by *P. falciparum*, the prevalence of *P. vivax* is faster than the prevalence of *P. falciparum* in many endemic regions (2). For the time being, although malaria-eliminating programs

are performing in 25 out of 32 countries in the world, these efforts merely or chiefly are against *P. vivax* (3). Due to the limitation of conventional microscopy, new direct diagnostic techniques have aroused the research for immunodiagnostic methods.

Existence of the malaria parasite in the organs of patient causes the creation of an extensive range of antibodies, both specifically against Plasmodia antigens and against nonspecific cells or agents such as red blood cells, leukocytes, rheumatoid factor, etc. (4). A few days after the invasion of the parasite into the bloodstream, species-specific, stage-specific and genus specific antibodies are identifiable and may persist long after the infection has occurred. There are antibodies against all blood stages of the parasite schizogonic cycle even against exoerythrocytic schizonts, but commonly available serological tests are aimed at the detection of antibodies against asexual blood stages for applicable intentions (supply of antigen) (5). The best antigens are obviously homologous antigens (*P. falciparum, P.vivax, P. ovale, P. malariae*), but an adequate supply is practically accessible merely for *P. falciparum*, which is cultured in vitro. For the serological diagnosis of the other species of Plasmodia, heterologous antigens are to be used (*P. cynomolgi* for *P. vivax* and *P. brazilianum* for *P. malariae*).

Merozoite surface protein 1 (*MSP-1*) is one of the most promising candidates among the antigens presently under examination in malaria vaccines and diagnostic kits (6). On the parasite surface, the *MSP-1* antigen is expressed as a large protein of 190–200 kDa. During the merozoite maturation, this precursor undergoes two phases of proteolytic cleavage. First, it is separated into four great fragments of 83, 30, 38 and 42 kDa (further referred to as *MSP-1*$_{83}$, *MSP-1*$_{30}$, *MSP-1*$_{38}$, and *MSP-1*$_{42}$), then the *MSP-1*$_{42}$ fragment undergoes a second cleavage before erythrocytic invasion which leads to the production of 33 and 19 kDa (*MSP-1*$_{33}$ and *MSP-1*$_{19}$) fragments, while during the invasion, only *MSP-1*$_{19}$ remains on the merozoite surface (7).

Seroepidemiology of malaria is evaluated by detection of antibodies using ELISA. However, regarding *P. vivax*, the difficulty of blood stage cultivation has prevented the use of this methodology. Making recombinant proteins via the methods of genetic engineering can provide adequate *P. vivax* blood stage antigens for the foundation of specific serological assays. Regarding the use of recombinant proteins established upon the sequence of the merozoite surface protein-1 (*MSP-1*) of *P. vivax* in the series of immuno-epidemiological studies, we inferred that, carboxy-terminal region of *MSP-1* recombinant protein was highly immunogenic that was recognized by antibodies of persons who was lately exposed to *P. vivax* (8). In addition, the carboxy-terminal region of *MSP-1* gene from *P. vivax* exhibits restricted polymorphism alleles (especially MSP-*1*$_{19}$) in various regions of the world, which does not restrict recognition by human antibodies (9, 10). Subsequently, these results suggest that it could be possible to develop serological tests using recombinant proteins based on the *P. vivax* carboxy-terminal $_{42}$ kDa region of *MSP-1*. This recombinant protein can be used in malaria serological diagnostic tests such as ELISA after serologic evaluation explained above.

The present study investigated whether recombinant *P. vivax* merozoite surface protein 1 (*PvMSP-1* $_{42}$ kDa) could be useful in detection of antibodies from the serums of a *P. vivax* infected person using serological tests.

## Materials and Methods

### *Preparation of insert DNA*

An Iranian infectious resident of Chabahar district (Sistan and Balouchestan Province) was chosen as the case. Its polymorphism parasitic target gene was the most common form among 50 infected persons that their target gene was recognized in this region. Using the EDTA tube, the patient's blood was collected and confirmed for *P. vivax* infection by microscopic examination, which was done utilizing Giemsa, stained thick and thin blood smears.

The parasite genomic DNA was extracted from the whole blood by P.C.I (phenol/chloroform-m/isoamyl alcohol) extraction method. Continuously, Genomic DNA quantity and quality was

evaluated by electrophoresis on 1% agarose gel and a biophotometer (Ependorf) at 260 and 280 nm. Amplification from 42 kDa partial regions of *PvMSP-1* gene was done which includes fragments 19 kDa and 33kDa. The primers were designed based on the sequence of *PvMSP-1* $_{42}$ kDa gene, (Genbank: Accession NO. DQ907673). The following primers were used for sequencing:
*MSP1.*$_{42}$F (25mer) (5'-GGATCCGACCAAGTAACAA-CGGGAG-3'),
*MSP1.*$_{42}$R (25mer) (5'-GAATTCCAAAGAGTGGCT-CAGAACC-3').
The PCR was performed in a PCR tube containing; 400 ng (1.5µl) extracted DNA as template, 30 pmol (0.5 µl ) of each primer, 15 µl of PCR master mix 2X that contained *Taq* and 12 µl of $ddH_2O$. The objective gene was amplified for 30 cycles (Initial denaturation at 96 °C for 5 min, 96 °C for 30 s, 58 °C for 30 s and 72 °**C** for 1 min and final extension for 20 min) subsequently, the PCR product was evaluated on 1% agarose gel against a standard DNA ladder (Fermentase Co.).

### *T-Vector preparation*

Any plasmid can be selected for T-Vector preparation which gratifies our requirements such as a plasmid containing a unique blunt-end restriction site in the multiple cloning site (in this study, the *EcoR V* site of the pTZ57R plasmid was used that has blue/white color selection).

### *Digesting plasmids with Blunt-End restriction enzyme*

Digesting plasmids were performed in a 0.5 ml microcentrifuge tube containing; 25 µl plasmid DNA (2µg), 4 µl of 10X *EcoR V* buffer, (10 unit) 2 µl of *EcoR V* (Fermentase Co.) and $ddH_2O$ up to 40µl. Then it was mixed by gentle vortex, centrifuged and incubated at 37 °C for 2 hours. Afterwards, 2 µl of the mixture was taken and run on a 1% agarose gel to make sure the digestion was completed. Then it was incubated to 65 °C by water bath for 10 min at the end of digestion (Fig. 1A). Blunt-ended plasmid DNA was purified by electrophoresis on 1% agarose gel.

### *Making T-overhangs from the Blunt-Ended plasmid vector*

*Taq* polymerase and dTTP were used to add a 3'-T to the blunt-ended plasmid. Briefly, the making T-overhangs was performed in a 200 µl PCR tube containing; 40 µl blunt-ended plasmid DNA, 5 µl of 10 X PCR buffer ($MgCl_2$ free), 2.5 µl of $MgCl_2$ (250 mM), 0.5 µl of dTTP (100 mM), 2 µl of *Taq* polymerase (5 U/µl). It was mixed by gentle vortex, centrifuged shortly and incubated at 72 °C for 2 h. The T-vectors were separated, purified and recovered on 1% agarose gel against a standard DNA ladder (Fermentase Co.).

### *Ligation*

Usually 50-60 ng of T-vector is enough for each ligation. 1:3 molar ratio of T-vector to insert DNA was recommended, but more than 2 µl of A-tailed DNA solution should not be added in a 10 µl ligation. In a sterile 0.5 ml microcentrifuge tube, added; 2 µl T-vector, 2 µl of A-tailed DNA , 1 µl of 10 X ligation buffer, 1 µl of T4 DNA ligase (2-3 U/µl) and 4 µl of $ddH_2O$. It was mixed gently, centrifuged briefly and incubated at 14 °C for overnight (Fig. 1B).

### *Transformation of the ligated vector*

Before transformation, Luria-Bertani (LB) agar plates contained 100 µg/ml of ampicillin, were prepared and spreaded with 100 µg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) and 1.0 mM isopropylthio-β-D-galactoside (IPTG). For each ligation reaction, 50 µl aliquoted of frozen competent cells (Top 10) was thawed on ice and added 2 µl of the ligation reaction to the competent cells and mixed gently by stirring with the pipette tip. The cells were incubated on ice for 30 min then heat shocked for 30s in the 42 °C water bath, and then were immediately placed on ice for 2 min. The transformed cells were spreaded on each labeled LB-ampicillin plate with X-Gal and IPTG. The plates were inverted and placed at 37 °C incubator for overnight. The positive colonies that have white color were identified by either restriction enzyme or screening by PCR method after transformation. PCR screening was

carried out with same primers that be used for PCR amplification for insert production. For screening using restriction enzyme method, the insert was released by digestion with two unique restriction enzymes (with cutting sites, *EcoR1*: GAA↓TTC and *BamH1*: GGA↓TCC) from the multiple cloning sites, and the insert size was confirmed by agarose gel electrophoresis (Fig. 1C). The recombinant plasmid was extracted (11) and digested by *EcoRI* and *BamHI* restriction enzymes. The released fragment DNA *MSP-1* $_{42}$ was ligated into pGEX 6P1 expression vector that digested by *EcoRI* and *BamHI* then, it was transformed in *E. coli*, BL21. Recombinant plasmid was confirmed by both colony PCR and enzyme digestion methods (12). PCR screening was carried out by same primers that be used for PCR amplification for insert production.

### Expression of recombinant protein

The *E. coli* strain BL21 contained r pGEX 6P1 was transformed and spread on LB (Luria Bertani) agar containing 100 µg/ml of ampicillin. The transformant was injected into 4 ml culture tube containing modified YT medium and allowed to grow at 37 °C in a shaker at 200 rpm, overnight (13). The next day, it was subcultured into two 10 ml flasks containing YT medium then, incubated at 200 rpm in a shaker at 37 °C. The culture in the logarithmic phase was induced by 1 mM IPTG for 3h, 5h and 8h. In this study, to achieve the highest expression at specified OD, suitable environment and temperature, induction with different amounts of IPTG (mM 0/5, 0/8, 1 and 1/5) in OD=600 (0/4 – 0/9) was done. Sediment bacteria was also tested at different times (3, 5 and 8h) after the induction. *MSP-1*$_{42}$ was expressed in inclusion bodies; therefore, the cell pellet was resuspended (from 20 ml of cultured bacterial) in 2 ml of inclusion body isolation solution (50 mM Tris–HCl, pH 8.0, 1 mM EDTA, 0.2 mg/ml lysozyme). The suspension was lysed on ice by sonication with 10 cycles, each consisting of 20 s pulses with 20s intervals. The bacterial lysate was centrifuged at 12000 × g for 15 min, and the supernatant was carefully discarded. The pellet was washed three times with inclusion body washing buffer (1 M urea, 0.5% Triton X-100, 50 mM Tris–HCl, pH 8.0, 1 mM EDTA). The inclusion bodies were collected by centrifugation at 12000 × g for 10 min. Inclusion bodies were dissolved in 2 ml solubilizing buffer (50 mM $NaH_2PO_4$, pH 8.0, 10 mM Tris–HCl, pH 8.0, 8 M urea, 100 mM NaCl) and vortexed until the pellet was completely resuspended and then incubated for 2h at room temperature. Afterwards, the sample was centrifuged at 23000 × g for 5 min to pellet any insoluble debris, the supernatant was transferred to the tube containing the resin (glutathione-sepharose 4B) and gently agitated at room temperature for 30 min. Sampling was done and analyzed on 12% (v/v) sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDSPAGE), and the gel was stained with Coomassie brilliant blue R-250 (14, 15).

### Protein purification

Induced bacteria by IPTG (1 mM) were grown massively for overnight at 37 °C. The C-terminal region of *PvMSP1* of *P. vivax* was expressed whit Glutathione S-Transferase (GST) fusion proteins. The detailed construction of these fusion proteins has been described elsewhere (16, 17). As a control, GST was produced lonely. Recombinant proteins and GST were affinity purified on glutathione-sepharose 4B columns (Pharmacia, Uppsala, Sweden) and their purity was determined by SDS-PAGE. The recombinant protein was purified by GST affinity chromatography according to the manufacturer's instructions and the protein concentration was measured (15 mg/L) by the Bradford assay with a spectrophotometer (Eppendorf, Germany).

### Western blot analysis

Proteins determined by SDS-PAGE were transferred to a nitrocellulose membrane electrophoretically. The membranes were incubated in TBS (Tris-Buffered Saline) containing 3% BSA (Bovine Serum Albumin) and then washed several times. The strips were reacted with human serum for 1 h at 37 °C. The membranes were washed several times with TBS and TBST and subsequently treat-

ed with horseradish peroxidase (HRP) conjugated rabbit anti human Ig at a 1:2000 dilution for 1 hour at 37 °C. The strips were visualized for color after development in $H_2O_2$ substrate solution for 15 min at room temperature. The reaction was stopped by washing four times in distilled $H_2O$ (18).

As mentioned Serological evaluation was carried out by Western blot analysis of the serum samples collected from inhabitant individuals in the endemic districts for malaria in south of Iran and compared with serum samples from persons never confronted from *P. vivax*.

## Results

DNA was extracted and the region of *MSP-1*$_{42}$ was amplified by PCR, cloned into pTZ57R/T vector and sequenced. R pTZ57R/ *MSP-1*$_{42}$ was digested by *BamHI* and *EcoRI* restriction enzymes and expected DNA band was released and recovered by P.C.I extraction method (Fig. 1). Then, insert ligated in to *BamHI* and *EcoRI* digested pGEX-6P1 for product r pGEX-6P1/ *MSP-1* $_{42}$, next Sub cloning of gene was confirmed by enzyme digestion via *BamHI* and *EcoRI* restriction enzymes (Fig. 2).

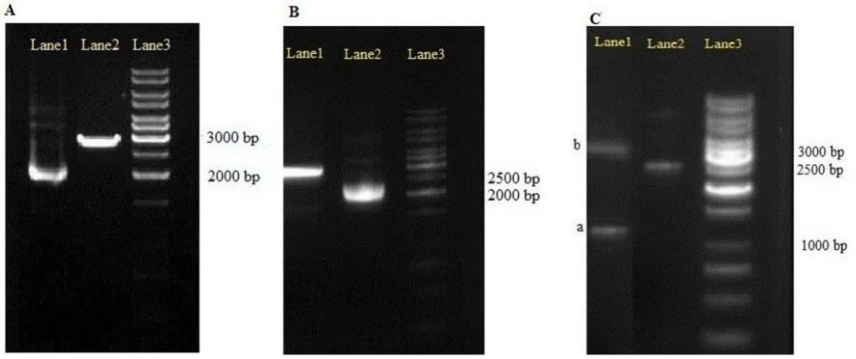


**Fig.1:** Intact plasmid pTZ57R, digested plasmid pTZ57R by *EcoRV* and digested recombinant pTZ57R/ MSP by *BamHI* and *EcoRI* restriction enzymes and expected insert band were shown. A: Lane 1: Intact plasmid pTZ57R, Lane 2: Digested plasmid pTZ57R by *EcoRV*, Lane 3: Size marker: 1kb. B: Lane 1: Recombinant plasmid, Lane 2: Intact Plasmid, Lane 3: Size marker: 1kb C: Lane 1: (a: Separated PCR product from digested recombinant plasmid, b: Digested recombinant plasmid ), Lane 2: Intact recombinant plasmid, Lane 3: Size marker: 1kb

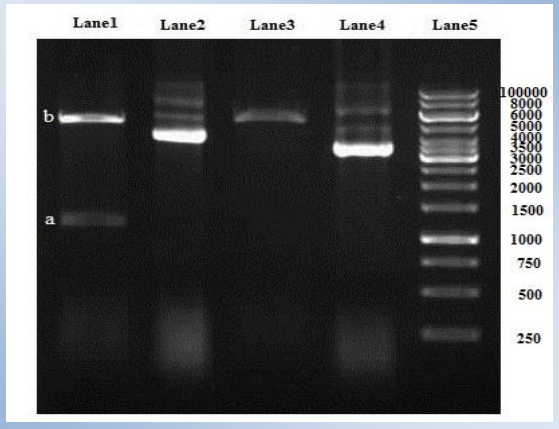


**Fig. 2:** Intact plasmid pGEX-6P1, digested plasmid PGEX-6P1 by *EcoRV* and digested recombinant pGEX-6P1/ *MSP-1* $_{42}$ kDa by *BamHI* and *EcoRI* restriction enzymes and expected insert band were shown. Lane 1: {a: Separated insert from digested recombinant expression plasmid (pGEX-6P1/ *MSP-1* $_{42}$ kDa) b: Digested recombinant expression plasmid}, Lane 2: Intact recombinant expression plasmid, Lane 3: Digested Intact expression plasmid, Lane 4: Intact expression plasmid, Lane 5: Size marker: 1kb

Finally, expression of *MSP-1*$_{42}$ using the BL21-pGEX-6P1 expression system, temperature of 37 °C, the induction with 0/5mM IPTG and precipitation 3 h after induction was very significant. Because of conjugated with GST (28 kDa), the r pGEX-6P1/ *MSP-1* $_{42}$ must be approximately 70 kDa molecular weights after induction on SDS-PAGE (Fig. 3). Recombinant proteins were expressed as GST fusion proteins and purified by GST affinity chromatography (Fig. 4). The GST affinity facilitates binding to glutathione sepharose. Western blotting was used to test the sensitivity and specialty of affinity-purified pGEX-6P1/ *MSP-1* $_{42}$, against the patient sera, sera from healthy individuals and sera from individuals with other infectious diseases. Western blots of affinity-purified pGEX-6P1/ *MSP-1* $_{42}$ demonstrated that a fusion protein reacted with patient's serum but not reacted with other infectious diseases and healthy individual's sera (Fig. 5).

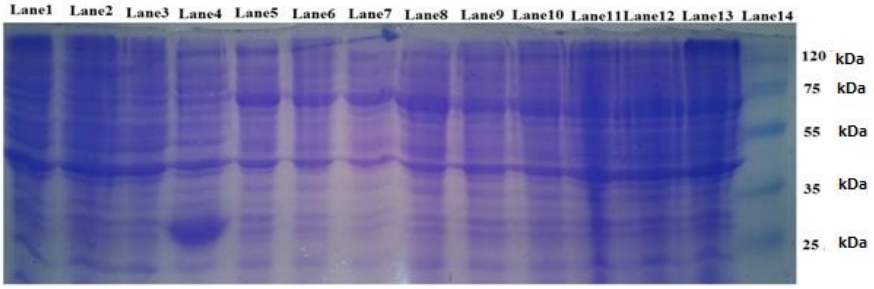


**Fig. 3**: SDS–PAGE analysis on the expression of pGEX-6P1/ *MSP-1* $_{42}$ kDa in *E. coli* (BL21). The amount of sample in each lane is 20 µl from 1 ml of cell culture. Samples were run on 15% polyacryl- amide–SDS gels. After electrophoresis, gel was stained with Coomassie blue. Lane 1, Cell lysate without plasmid( *E. coli* - BL21) induced with IPTG; lane 2, lysate of bacteria containing pGEX-6P1 /BL2 induced with IPTG 1; lane 3, lysate of bacteria containing pGEX-6P1- *MSP-1* $_{42}$ kDa /BL21 without induced with IPTG; lane 4, Lysate of bacteria containing pGEX-6P1 /BL21 after induced with IPTG; lane 5,6 and 7, Lysate of bacteria containing pGEX-6P1-*MSP-1* $_{42}$ kDa /BL21 collected 3h cell culture after induced with IPTG; lane 8,9 and 10, Lysate of bacteria containing pGEX-6P1-*MSP-1* $_{42}$ kDa /BL21 collected 5h cell culture after induced with IPTG; lane 11,12 and 13, Lysate of bacteria containing pGEX-6P1-*MSP-1* $_{42}$ kDa /BL21 collected 8h cell culture after induced with IPTG; Lane 14, molecular standards.

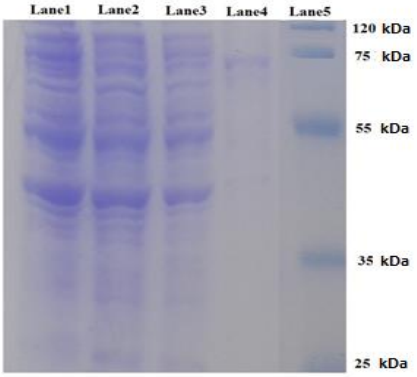


**Fig. 4 :** Different stages of recombinant protein purification via GST/affinity chromatography method for assessing rate of purification. Samples were run on 15% polyacryl- amide–SDS gels. After electrophoresis, gel was stained with Coomassie blue, Lane 1: lysate of pGEX-6P1- *MSP-1* $_{42}$ kDa /BL21 collected 3h cell culture; Lane 2 and 3: the washing solution was passed through the glutathione sepharose column; Lane 4: elution buffer with purified protein released from GST resin without concentration; Lane 5: molecular standards

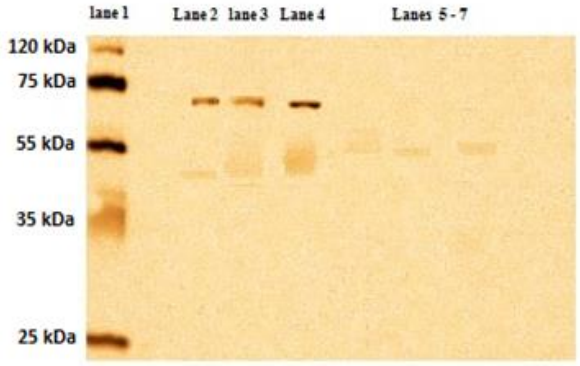


**Fig.5:** Western blot analysis of GST/ *MSP-1* $_{42}$ kDa with different antigen concentrations against patient sera. Samples were run on 15% polyacryl- amide–SDS gels and then transferred to nitrocellulose/Lane1: Protein size marker, Lane2: GST/ *MSP-1* $_{42}$ kDa without concentration, Lane 3 and 4: GST/*MSP-1* $_{42}$ kDa with 2X and 4X concentration reacted with a same human *P.vivax* positive serum (1:100 dilution) , Lane5-7: purified GST that not reacted with human *P.vivax* positive serum

**Table 1:** Western blotting results based on recombinant protein of *P. vivax* (*MSP-1*$_{42}$kDa)

| Numbers / Samples | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| sera from naturally infected individuals | + | + | + | + | + | + | + | + | + | + | 10 |
| sera from healthy individuals | - | - | - | - | - | - | - | - | - | - | 10 |
| sera from individuals with other infectious diseases | - | - | - | - | - | - | - | | | | 7 |
| Total sera samples | | | | | | | | | | | 27 |

Note. Naturally infected individuals means individuals infected with *Plasmodium vivax* that verified by microscopic examination. Healthy individuals means people who have lived in non-endemic areas and they had no contact with the parasite during their lifetime and being healthy proved by microscopic examination. individuals with other infectious diseases means people who were infected with other diseases such as toxoplasmosis, visceral leishmaniasis, hydatid cyst, fever of unknown cause, and 3 sera from individuals infected with *Plasmodium falciparum*

## Discussion

In several conditions, an influential technique to carry out a precise diagnosis of malaria infection is very important, not only in endemic countries, but also for epidemiological, clinical and research goals. In non endemic countries, usually occurrence of clinical symptoms is the cause which encourages the patient to confer to the physician. Therefore, clinicians should have efficient knowledge of the probability of imported malaria cases. Whereas, the exposure to the infection might have been a long time before clinical symptoms start to show, therefore diagnosis in such cases could be difficult, especially in *P. vivax* and *P. malariae*. On the contrary, in malaria endemic countries due to economic and logistic reasons, entire populations rely on self-diagnosis and treatment solely, even in the cases of moderate fever. But unfortunately, the mild clinical symptoms of moderate malaria are very general (headache, fever, myalgia, etc.), which is similar to a vast series of other clinical cases. In highly endemic areas wherever the laboratory confirmation of malaria is not available the experienced clinician may recognize malaria with adequate predictive positive worthwhile and positive blood slide (19).

As malaria is seen on clinical cases, it is compulsory to acquire the laboratory confirmation. Distinguishing malaria can be pursued by the exhibition of the parasite's whole cell, parasite's nucleic acid and the products in the blood (direct diagnosis) or by the exhibition of the patient's immune response to the infection (indirect diagnosis or immunodiagnosis). The foundation of malaria assessment was initiated when Laveran observed and described malaria parasites in human blood thereupon direct diagnosis of malaria infection has been established. This method is still golden standard. For the majority of clinical conditions, conventional microscopy is usually acceptable, but may be unsuitable for very low parasitaemia (below the detection threshold of 10 parasites/μl of blood) and to find drug-resistant parasites. Thus modern molecular biologic methods for conquest these barriers has been explored.

Based on the parasite's repetitive Plasmodium genome as the diagnostic target, diagnosis researches in nucleic acid-based malaria were initiated (20). Hence, two small subunits (18S) rRNA genes from *P. vivax* were sequenced and species-specific regions of the rRNA genes were operated in developing sensitive diagnostic procedures (21, 22). Nevertheless, in the use of PCR for detection of malaria infection may be a number of barriers such as contamination, false positive results, and high costs exist. Accurate and expensive precautions (infrequently accessible in field conditions) are required to reduction the measure of contamination of PCR with previously amplified products (23).

Lactate dehydrogenase (product in the blood) has been assessed as a method of quantifying parasitaemia and drug resistance for discernment of

blood levels of parasite-specific (24). This method can be automated allowing the processing of large numbers of samples, but its sensitivity has proven to be quite low (25). The researches have stimulated for immunodiagnostic techniques because of the restriction of conventional microscopic method (low sensitivity, trouble intensive, well-trained personnel) and the new diagnostic direct techniques (advanced measuring equipment) that could process a large amount of samples in a standardized way. Immunofluorescence assay (IFA) was used for diagnosis malaria antibodies as the first serological test, which can be used for both G and M specific immunoglobulins. Its sensitivity and specificity rely on the technician's expertise. The indirect hemoagglutination test (IHA) is convenient for field studies, but its specificity and sensitivity are weak. The enzyme-linked immunosorbent assay (ELISA) has similar specificity and sensitivity specifications like the IFA test, but the explanation of its results can be better standardized.

High plentifulness and important function on the cell surface, probably caused *MSP1* to be a principal object of the host immune system and antibodies identified different regions of this protein. Antibodies that recognize the C-terminal region of *P. falciparum MSP-1*, inhibit invasion of the merozoites into the host erythrocytes in vitro and immunization of experimental animals with *MSP-1*$_{19}$ kDa confers protective immunity (26).

As vaccine candidates, recombinant antigens of different asexual blood stage parasites have been recognized and studies on the serum of infected patients with Plasmodium has shown the antibody titers against them (27 - 30). Also some studies used *PvMSP-1* potential as malaria vaccine candidate, in these studies the C-terminal region of the protein (*MSP-1*) has been detected in Plasmodia and it was shown that its activity was blocked by specific antibodies. For instance, Soares et al. (31) evaluated the N and C-terminal regions of *MSP-1* recombinant protein, which were expressed in *E.coli* separately to detect specific immune responses. They reported that the recombinant protein which based on the 19-kDa C-terminal region of *PvMSP1* (*PvMSP-1*$_{19}$) contains the two epidermal growth factor (EGF)–like regions, was the most immunogenic and the antibodies of 83.8% of the individuals recognized this recombinant protein. Furthermore, the antibody titers to the C-terminal region of PvMSP-1 were higher than the N-terminal region.

In another study, the N and C-terminal regions of MSP-1 recombinant protein, which were expressed separately in *E.coli* , generated cellular and humoral immunity in mice (32) .

Cunha and colleagues (33) expressed the *MSP-1* $_{19}$ kDa in pET, pGEX and pMAL vectors and investigated the rate of their immunization in mice as well as the ability to detect antibodies in the patients. They indicated that the power of mice immunization via recombinant *MSP* $_{19}$ kDa, in pET and pGEX vectors is more than pMAL vector. On the other hand, the power of antibody detection via resent recombinant protein that expressed in pET vector is better than pGEX vector.

In Sachdeva et al.'s study (34), *MSP-1* $_{19}$ *kDa* and *MSP-1* $_{42}$ kDa recombinant proteins which were expressed separately in *E.coli,*, were evaluated for immunization and assessment of the diagnostic response in the host. They reported that both recombinant proteins can induce $IgG_1$ in the host, $IgG_2$ and $IgG_3$ subsequently. They also stated that , *MSP* $_{42}$ kDa is better to activate cellular and humeral immunity than the *MSP* $_{19}$ kDa due to either being bigger or having more epitopes in the N-terminal or C-terminal portion containing the two epidermal growth factor (EGF)–like regions.

The C-terminal epitopes of *MSP-1* $_{19}$ recombinant protein that expressed by pET vector is capable of activate T cells and induces the production of IgG in the host (35).

The C-terminal region of both *MSP-1* $_{19}$ and *MSP-3a* recombinant proteins are capable of detecting different levels of IgG sub-classes in infected sera and they reported *MSP* $_{19}$ relative to the *MSP-3a* to be much more protective and immunogenic (36).

In all the above mentioned studies, it was pointed out that in malaria, the acquired immunity is specific and depends on species and strains in each country. Therefore, global studies may not be fully responsive to the specific needs of each region. In any geographic region, specific and detailed studies should be performed according to the local parasites. This fact that, in this study, all serum samples reacted positively by Western blot examination indicates that expression system for refolding the disulfide bonds of spatial domain in r *Pv* $MSP_{42}$kDa has operated successfully and simulated with native *Pv* $MSP_{42}$kDa.

These findings demonstrate that *MSP-1* $_{42}$ is a promising candidate antigen for blood stage vaccine development and producing of the diagnostic kits. Serological diagnosis of *P. vivax* patients has been exactly performed by applying of recombinant proteins because of difficulty in culturing blood stages in *P. vivax*. In the current study, we cloned *MSP-1* $_{42}$ gene and purified recombinant protein produced from *E. coli* , BL21 which was expressed by pGEX 6P1 vector, then its ability to be identified by IgG antibodies of 10 individuals who were contaminated with *P. vivax* infection was investigated. Recombinant protein was confirmed by using SDS-PAGE. Western blot analysis was carried out by human serum to confirm the expressed protein in *E. coli* cells after induction with 0.5 mM IPTG for 3h. The Glutathione S-Transferase (GST) fusion protein in the pGEX series was purified by gluthatione Sepharose 4B.

## Conclusion

For the first time in Iran, we successfully cloned and highly expressed *MSP-1* $_{42}$ in pGEX 6P1 , which can be used in malaria immunodiagnostic tests. Tests with 10 specific and cross-reactive sera were carried out and antigen diagnostic value was confirmed. The antigens were provided from prevalent parasite strains in Iran (37, 38) which by doing so the detection accuracy and efficiency will be promoted and this will be more beneficial compared to the ready-to -use kits imported from other countries.

## Ethical considerations

Tehran university of medical sciences of ethics committee approved the study protocol and all women was informed about the purpose of the study; what was involved in participating; confidentiality and anonymity issues; and, the right to withdraw at any time without repercussions. Following their approval to participate the interview, their consent, verbal or written, to record the interview was obtained.

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