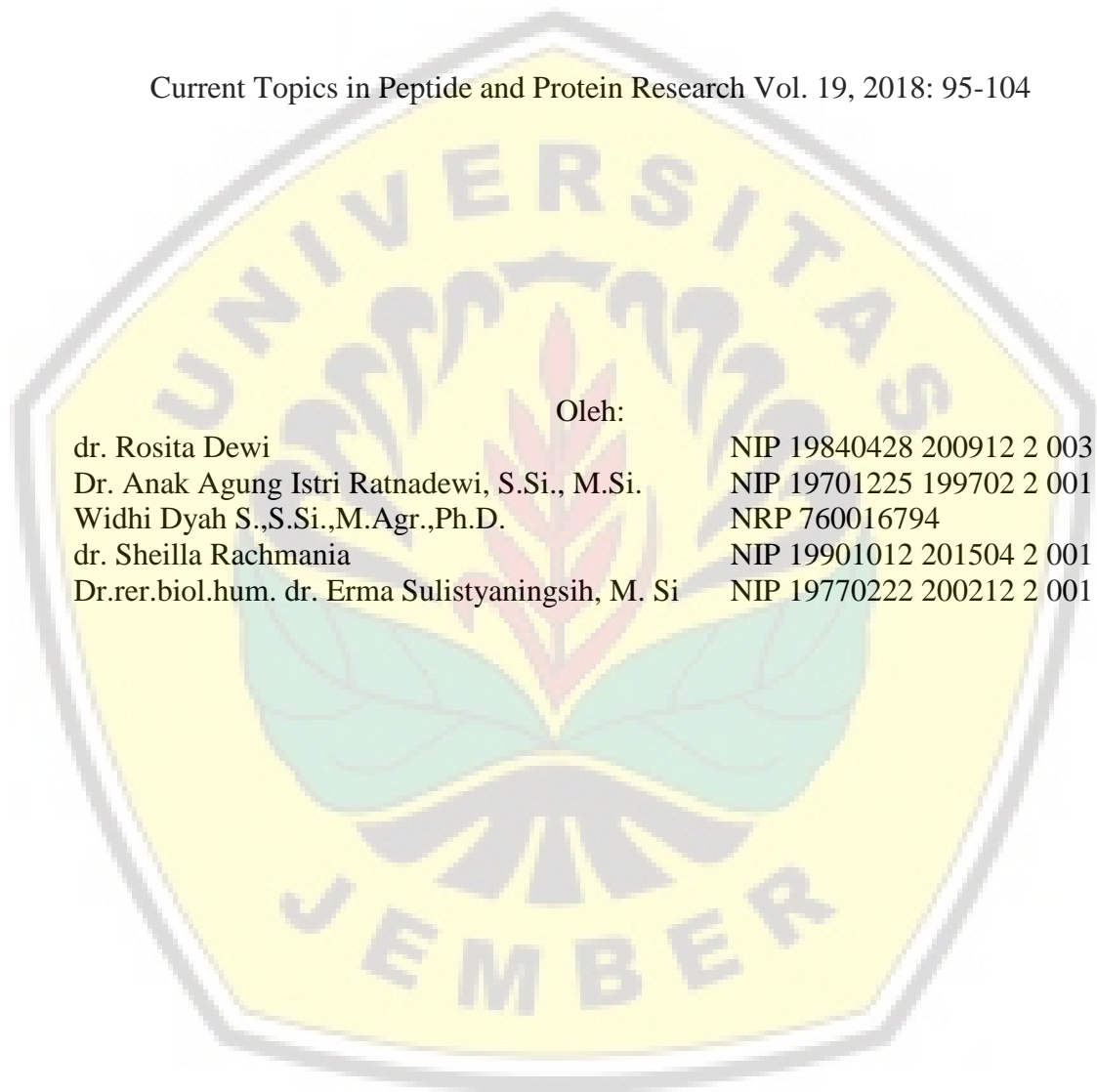


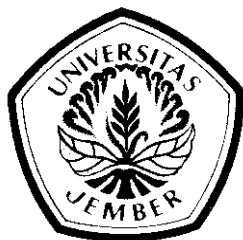
**Cloning, Sequence Analysis, and Expression of CIDR1 α -PfEMP1
from Indonesian *Plasmodium falciparum* Isolate**

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**KEMENTERIAN RISET, TEKNOLOGI, DAN
PENDIDIKAN TINGGI
UNIVERSITAS JEMBER
FAKULTAS KEDOKTERAN**

Cloning, sequence analysis, and expression of CIDR1 α -PfEMP1 from Indonesian *Plasmodium falciparum* isolate

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ABSTRACT

Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is an antigenic protein which is expressed in the erythrocytic phase of *P. falciparum* life cycle and plays an essential role in severe malaria pathogenesis. It is encoded by the *var* gene family and consists of cysteine-rich interdomain region (CIDR) and Duffy-binding-like (DBL) domains. This research aimed to clone, analyze, and express the CIDR1 α -PfEMP1 from Indonesian *P. falciparum* isolate. The specific CIDR1 α domain was amplified from malaria patients. The amplicon was ligated into pET-30a and transformed into *Escherichia coli* BL21(DE3). Sequencing result of plasmid clones confirmed 100% homology with the sequence of the CIDR1 α domain. BLAST analysis showed that the sequence had high similarity to *Plasmodium sp. gorilla clade G1* from Africa and *P. falciparum* isolate from Tanzania, East Africa. Translation using ExPASy Translate Tool resulted in a truncated protein consisting of 99 amino acids. Western blot analysis using anti-His-6-tag antibody showed that the recombinant protein was a 17.5 kDa fusion protein and clearly expressed as a soluble protein when induced with 0.1 mM isopropylthio- β -galactoside (IPTG) for ≥ 8 hours at room temperature. As much as 45.4% of amino acids showed 100% homology with other human-infecting

P. falciparum isolates. The sequence was predicted to have B-cell epitope based on immune epitope database. In conclusion, Indonesian CIDR1 α -PfEMP1 isolate had high similarity to primate and human-infecting *Plasmodium spp.* isolates. The recombinant protein was expressed as a fusion protein. Its structure prediction showed that the sequences have B-cell epitope, implicating its potency in inducing antibodies. Further studies should be established to develop its potency as a vaccine candidate.

KEYWORDS: CIDR1 α , malaria, PfEMP1, *Plasmodium falciparum*, *var* gene.

INTRODUCTION

Malaria is one of the major health problems worldwide and the most significant health challenge in developing countries. According to WHO, in 2016 an estimated 216 million cases of malaria occurred worldwide with 445,000 deaths. Most of the cases were in the African Region (90%), followed by South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). Between 2014 and 2016, the incidence rate of malaria cases remained unchanged globally and increased in all WHO regions except for the WHO European Region [1]. Malaria incidence in Indonesia was 1.9% in 2013. Most cases were found in the Eastern part of Indonesia such as Papua, West Papua, East Nusa Tenggara, Maluku, and North Maluku [2].

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Plasmodium falciparum is the species that is responsible for most of the malaria cases. It is also the most virulent species among the five human-infecting *Plasmodium spp.* which is correlated with almost every malarial death [3]. This species can cause severe malaria due to the pathogenesis which involves the attachment of infected erythrocytes to vascular endothelial cells, termed as cytoadherence, resulting in microvascular obstruction of many vital organs [4, 5]. In addition, the parasite induces the attachment of infected erythrocytes to other uninfected erythrocytes, termed as rosetting, which further obstructs microvascular blood flow [4, 6].

Cytoadherence and rosetting are mediated by *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), an antigenic protein encoded by the *var gene* family consisting of approximately 60 variable genes. The gene consists of two exons. Exon 1 is highly polymorphic and encodes for the trans-membrane domain and an extracellular part of protein composed of N-terminal segment (NTS) domain, Duffy-binding-like (DBL) domains (α , β , γ , δ , ϵ , and χ), and cysteine-rich interdomain region (CIDR) domains (α , β , and γ). Exon 2 is more conserved and encodes for an intracellular acidic terminal segment (ATS) [7-10].

CIDR1 α domain mediates the binding to many host receptors such as cluster of differentiation 36 (CD36), cluster of differentiation 31 (CD31), and chondroitin sulfate A (CSA) to facilitate cytoadherence process, while the binding to CD36 and immunoglobulin M (IgM) mediates rosetting process leading to severe malaria [6, 9, 11, 12]. A previous study reported that CIDR domain as a part of N-terminal of PfEMP1 has a binding capacity to endothelial protein C receptor (EPCR). The interaction with EPCR provokes pathological processes such as coagulation, vascular inflammation, and an increase in endothelial permeability, which have significant contributions in cerebral malaria symptoms [13]. In this study, we cloned, analyzed, and expressed the CIDR1 α -PfEMP1 from Indonesian *P. falciparum* isolate as an effort to support malaria control program.

MATERIALS AND METHODS

Recruitment of samples and research site

Blood samples were collected from malaria patients who enrolled in the Primary Health Care in Jember

district, Indonesia in 2014. The patients were informed about the study and signed the informed consent. This study was approved by the Ethical Committee for Research, Faculty of Medicine, University of Jember, Jember, Indonesia with reference number 454/H25.1.11/KE/2014 and 1.114/H25.1.11/KE/2017.

Cloning of gene

CIDR1 α domain was amplified using HotStar Taq Master Mix Kit with a specific primer designed based on reference [7] with modification in restriction enzyme site. The primers used in this study were CIDR1 α _Fw 5'-CGGGATCCAAATGGAAATGTTATTATG-3' carrying a *BamHI* site and CIDR1 α _Rev 5'-CCCTCGAGTTGTAGTAATTATCAATT-3' carrying an *XhoI* site. Polymerase chain reaction (PCR) product was purified and ligated into the expression vector (pET-30a (Novagen)) after double-digested using *XhoI* (Thermo Scientific) and *BamHI* (Promega), and further transformed into *Escherichia coli* BL21(DE3) (Novagen) using the heat shock method [8].

Confirmation of cloning was performed by colony PCR using CIDR1 α _Fw primer and CIDR1 α _Rev primer, double digestion of plasmid clones using both *BamHI* and *XhoI*, and further *XbaI* and *XhoI*, and sequencing on both strands using The BigDye Terminator v3.1 cycle sequencing kit in ABI PRISM 3730 Version 3.1 sequencer (Applied Biosystems).

Sequence analysis

Nucleotide sequence similarity was analyzed using nucleotide Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and aligned using DNASIS MAX 3. Nucleotide sequences were translated into amino acid using ExpASY Translate Tool (<https://web.expasy.org/translate/>), analyzed using protein BLAST NCBI, and subsequently aligned using ClustalW Multiple Alignment in BioEdit program. The molecular weight of recombinant protein and amino acid composition were determined using ExpASY ProtParam Tool (<https://web.expasy.org/protparam/>).

Expression of CIDR1 α -PfEMP1 recombinant protein in *E. coli* BL21(DE3)

We combined the methods described in references [7, 12, 14] to express CIDR1 α -PfEMP1 recombinant

protein. The transformed *E. coli* BL21(DE3) were grown overnight in 2.5 mL of LB broth containing 50 μ g/mL kanamycin at 37 °C with shaking at 150 rpm. The overnight culture was inoculated into 10 mL of LB broth medium. When OD₆₀₀ of the culture was 0.5-0.6, as much as 0.1 mM isopropyl- β -D-thiogalactoside (IPTG) (Promega) was added, and the cells were harvested at 0 h, four hours, eight hours, and overnight at room temperature with shaking at 180 rpm.

For total protein, 1 mL of each culture was pelleted by centrifugation at 10,000 rpm for 2 min at 4 °C, added with aquadest and 2X sample buffer. For soluble and insoluble protein, each culture was pelleted by centrifugation at 6,000 rpm for 10 min at 4 °C. Bacterial cells were suspended in extraction buffer, lysed with 1 mg/mL lysozyme for 15 min and sonicated on ice, and centrifuged at 12,000 rpm for 20 min at 4 °C. Supernatant and pellet were added with 2X sample buffer in a 1:1 ratio and diluted five times using extraction buffer and 2X sample buffer. All samples were transferred into 95 °C dry heat block for 5 min [15].

Western blot analysis

Total, soluble, and insoluble proteins were run in sodium dodecyl sulfate (SDS) poly-acrylamide gel (PAGE) containing 15% acrylamide/bis, 4X lower gel buffer (1.5 M Tris-HCl pH 8.8 and 0.4% SDS), 10% APS, and TEMED, with 1X running buffer containing 0.025 M Tris base, 0.1% SDS, and 0.192 M glycine. The gel was electroblotted onto Immobilon-P transfer membrane (Millipore) for immunodetection using anti-His-6-tag antibody from rabbit serum (Bioacademia) against epitope contained in the tag. The proteins reacting with antibodies were visualized with alkaline phosphatase-conjugated goat secondary antibodies anti-rabbit IgG (BioRad) using the 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT) for color development [15].

B-cell epitope prediction of CIDR1 α -PfEMP1 recombinant protein

B-cell epitope prediction of CIDR1 α -PfEMP1 recombinant protein was performed using Immune Epitope Database and Analysis Resource (<http://www.iedb.org>). BepiPred predicted the location of linear B-cell epitope based on sequence

characteristics using a combination of a hidden Markov model and a propensity scale method.

RESULTS

We collected blood samples from a patient with clinically severe malaria presenting severe anemia (Hb 3 g/dL) and 15 patients with clinically uncomplicated malaria caused by *P. falciparum*. Amplification using specific primers showed a positive band only from a severe malaria sample. This case was an imported case from an area categorized as high endemic malaria areas in Indonesia.

Confirmation of cloning

Colony PCR

Cloning of gene yielded colonies which were directly confirmed by colony PCR. Gel electrophoresis of colony PCR products confirmed that a single band of approximately 520 bp resembled the previous PCR product.

Digestion of plasmid clones

Gel electrophoresis of plasmid clones digested using *Bam*HI and *Xho*I resulted in two bands of approximately 5,400 bp and 520 bp. The second double digestion of plasmid clones using *Xba*I and *Xho*I resulted in two bands of about 5,200 bp and 700 bp, as presented in Fig. 1. The digestion results confirmed the successful cloning process.

Sequencing

Sequencing of CIDR1 α -PfEMP1 amplicon yielded 526 nucleotides. Sequencing of plasmid clones showed that the sequence of the domain in three plasmid clones (pET_CIDR α 1, pET_CIDR α 2, pET_CIDR α 3) were identical to each other and indicated 100% homology with the sequence of a CIDR1 α -PfEMP1 amplicon.

Sequence analysis

Nucleotide sequence analysis

BLAST analysis of 526 nucleotides of CIDR1 α -PfEMP1 showed similarity to the CIDR1 α domain of other 20 *Plasmodium spp.* isolates infecting primates and humans. The three highest similarities to *Plasmodium spp.* infecting primates were found in *P. sp. gorilla clade G1* (LT963417.1) with 83%

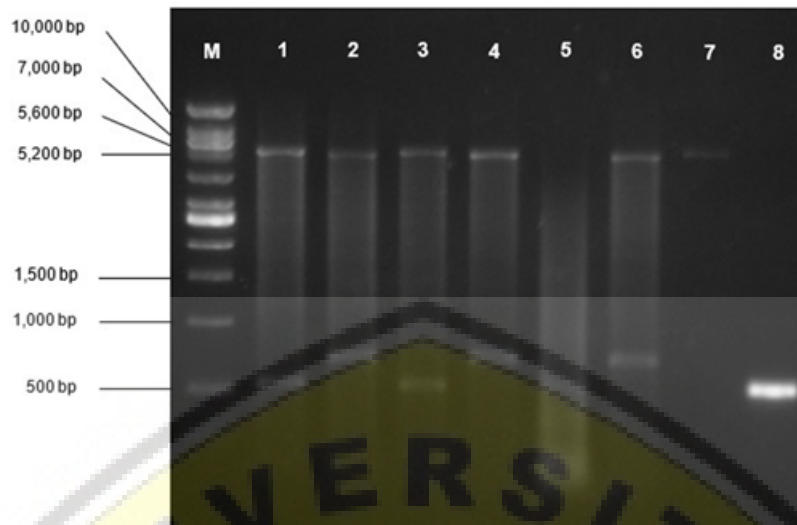


Fig. 1. Gel electrophoresis of double-digested plasmid clones. M: 1 kb DNA ladder; 1, 3, 5: pET_CIDR α 1, pET_CIDR α 2, pET_CIDR α 3 double-digested using *Bam*HI and *Xho*I; 2, 4, 6: pET_CIDR α 1, pET_CIDR α 2, pET_CIDR α 3 double-digested using *Xba*I and *Xho*I; 7: purified pET-30a double-digested using *Xho*I and *Bam*HI; 8: purified CIDR1 α -PfEMP1 amplicon double-digested using *Xho*I and *Bam*HI.

similarity in 99% coverage, *P. reichenowi* (LT969567.1) with 82% similarity in 89% coverage, and *P. billcollinsi* (LT990237.1) with 82% similarity in 71% coverage. The three highest similarities to other *P. falciparum* isolates infecting humans were found in *P. falciparum* isolate 1994-3 from Tanzania, East Africa (KX154905.1) with 85% similarity in 62% coverage; *P. falciparum* isolate 1734-2 from Tanzania, East Africa (KX154955.1) with 78% similarity in 99%, and *P. falciparum* 3D7 (XM_001350899.1) from Africa with 74% similarity in 98% coverage. Multiple alignments of the sequences with other isolates are presented in Fig. 2.

Amino acid sequence analysis

The translation of 526 nucleotides resulted in a truncated protein consisting of 99 amino acids. BLAST analysis of amino acid sequence demonstrated that the recombinant domain matched for CIDR1 α -PfEMP1 and had high similarities to primate-infecting *Plasmodium spp.* isolates such as *P. sp. gorilla clade G1*, *P. reichenowi*, and *P. billcollinsi*; and other human-infecting *P. falciparum* isolates such as *P. falciparum* Tanzania 2000708, RAJ116, NF135/5.C10, 1994-3, and UGT5.1. Multiple amino acid sequence alignment showed that cysteine conserved areas were located on N-terminal, H1-H2 loop, and H2 helix and our sequence

was probably composed of N-terminal, H1 helix, H1-H2 loop, and H2 helix, as presented in Fig. 3., based on the topology diagram of CIDR MC179 [10]. As many as 43.4% of amino acids showed 100% homology when our sequence was aligned with primate-infecting *Plasmodium spp.* isolates and 45.4% of amino acids showed 100% homology when our sequence was aligned with other human-infecting *P. falciparum* isolates.

Analysis using ExPASy ProtParam Tool showed that the recombinant protein had a molecular weight of 17.5 kDa. It consisted of 50 amino acids from start codon to the sequence of histidine tag followed by 99 amino acids of a CIDR1 α domain. Amino acid composition of recombinant protein is presented in Table 1.

Expression of CIDR1 α -PfEMP1 recombinant protein in *E. coli* BL21(DE3)

CIDR1 α -PfEMP1 recombinant protein was clearly expressed in the supernatant as a soluble protein when induced with 0.1 mM IPTG for ≥ 8 h and the pellet as an insoluble protein, when induced with 0.1 mM IPTG for ≥ 0 h. Those were visualized as a 17.5 kDa protein band, as showed in Fig. 4. Longer incubation time with IPTG increased the protein expression.

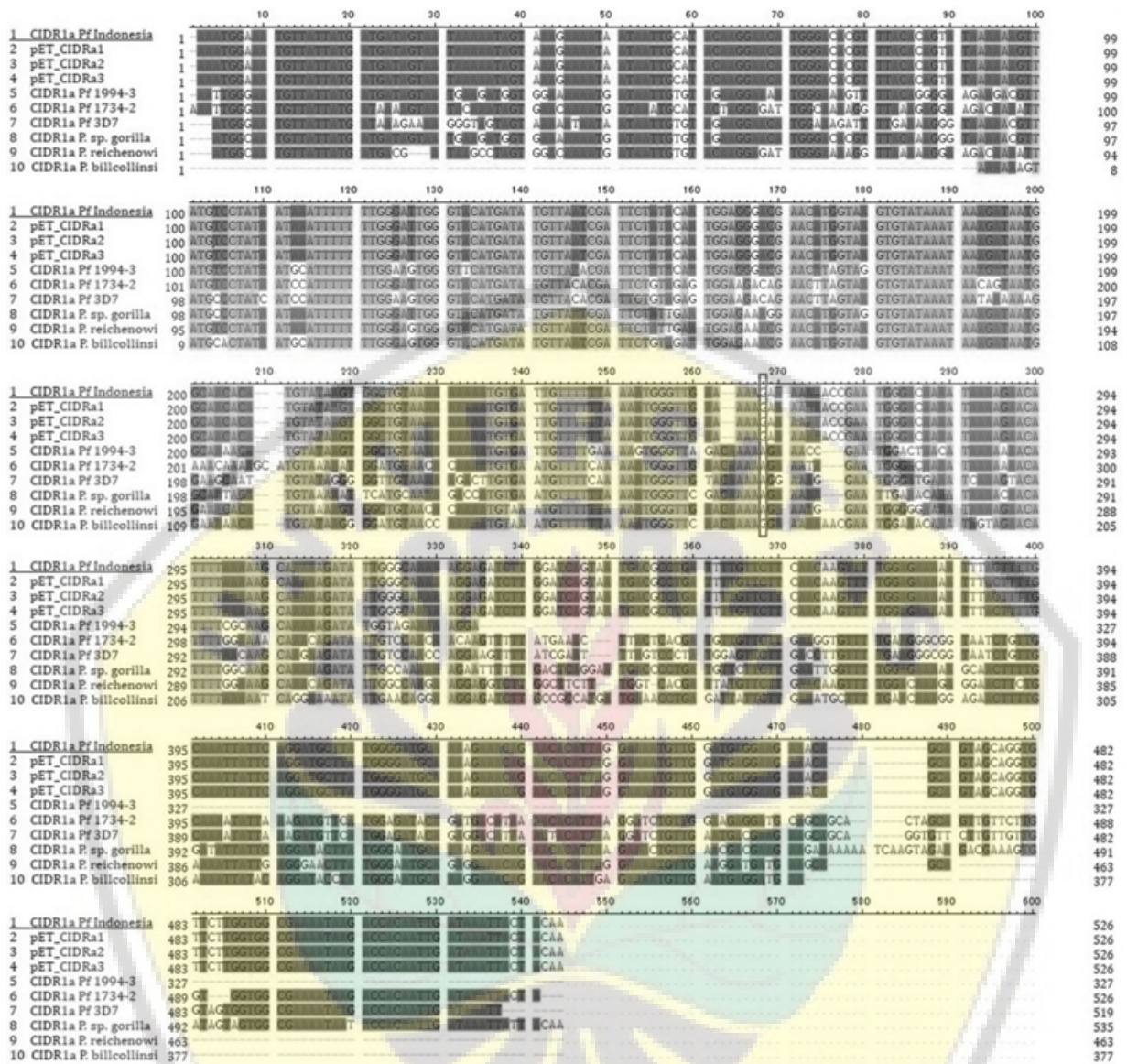


Fig. 2. Multiple nucleotide sequence alignment of CIDR1 α -PfEMP1. Row 1: CIDR1 α domain from Indonesian *P. falciparum* isolate; Row 2-4: CIDR1 α domain in plasmid clones; Row 5-7: Human-infecting *P. falciparum* isolates (KX154905.1, KX154955.1, XM_001350899.1, respectively); Row 8: Gorilla-infecting *Plasmodium* sp. (LT963417.1); Row 9-10: Chimpanzee-infecting *Plasmodium* spp. (LT969567.1, LT990237.1, respectively). Gray highlights indicate 100% homology. A black rectangle presents nucleotide 262 in the sequence of a CIDR1 α domain.

B-cell epitope prediction of CIDR1 α -PfEMP1 recombinant protein

The prediction of a B-cell epitope of CIDR1 α -PfEMP1 recombinant protein was performed using the Immune Epitope Database and Analysis Resource (<http://www.iedb.org>). Residues with scores above the threshold (default value: 0.35) were predicted to be part of a B-cell epitope, as presented in Table 2.

DISCUSSION

CIDR1 α -PfEMP1 from Indonesian *P. falciparum* consisted of 526 nucleotides which were similar to the CIDR domain of *P. falciparum* ItG2.F6 strain reported in a previous study, which resulted in an amplicon of approximately either 510, 520, or 600 bp [7]. The domain was involved in the pathogenesis of severe malaria by mediating adhesion of infected

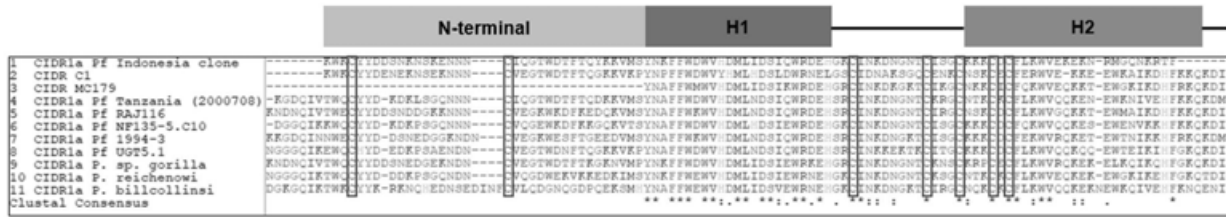


Fig. 3. Multiple amino acid sequence alignment of CIDR1 α -PfEMP1. Row 1: CIDR1 α -PfEMP1 from Indonesian *P. falciparum* isolate; Row 2: CIDR C1 started with N-terminal [7]; Row 3: CIDR MC179 started with H1 helix [10]; Row 4-8: human-infecting *P. falciparum* isolates (ETW33171.1, KNC35078.1, ETW44291.1, ANJ21025.1, EWC79317.1, respectively); Row 9: gorilla-infecting *Plasmodium sp.* (SOS76843.1); Row 10-11: chimpanzee-infecting *Plasmodium spp.* (SOV76311.1, SPJ09814.1, respectively). Black rectangles present conserved cysteine areas.

Table 1. Amino acid composition.

Amino acid	Percentage
Ala (A)	3.4%
Arg (R)	3.4%
Asn (N)	7.4%
Asp (D)	10.7%
Cys (C)	4.7%
Gln (Q)	3.4%
Glu (E)	4.0%
Gly (G)	6.7%
His (H)	6.0%
Ile (I)	4.0%
Leu (L)	2.7%
Lys (K)	12.8%
Met (M)	4.7%
Phe (F)	4.0%
Pro (P)	1.3%
Ser (S)	6.7%
Thr (T)	4.7%
Trp (W)	4.0%
Tyr (Y)	2.7%
Val (V)	2.7%

erythrocytes to several receptors such as CD36. It was reported that six recombinant CIDR domains could bind to CHO-CD36 cells in a dose-dependent manner as confirmed by fluorescence-activated cell sorting (FACS) analysis [7]. Another study showed that CIDR1 α domain bound to ELISA plates which are pre-coated with human CD31 and IgM [12] and immunization of mice with CIDR1 α domain

increased antibodies recognizing PfEMP1 and specifically inhibited CD36 binding [14]. A further study reported that crystallized CIDR1 α domain was able to bind to CD36 in the human CD36-transfected mammalian cell [10]. The proportion of *var* transcripts encoding CIDR1 α predicted to bind to EPCR was higher in children with severe malaria than children with uncomplicated malaria (median percentage of 54.1 vs. 7.4%) [16].

Nucleotide BLAST analysis indicated that our sequence had a close evolutionary relationship with gorilla-infecting *Plasmodium sp.* (*P. sp. gorilla clade G1*). It is widely known that *P. falciparum*, humans, and our forebears influenced each other in the process of development or evolution, termed as co-evolution, million years ago. Many studies have reported that the discovery of *Plasmodium spp.* infecting gorillas and chimpanzees has disputed the co-evolution theory and subsequently reported that *P. falciparum* is derived from parasites infecting wild-living African apes. Characterization study of the diverse ape *Laverania spp.* discovered the pedigree of *P. praefalciparum*, gorilla-infecting *Plasmodium sp.*, which was almost indistinguishable from *P. falciparum* [17]. Therefore, it is now agreed that *P. falciparum* has emerged from the cross-species transmission of the gorilla-infecting parasite [18]. A close evolutionary relationship of our sequence with other human-infecting *P. falciparum* isolate was found in *P. falciparum* isolate 1994-3 from Tanzania, East Africa (KX154905.1) [16]. In addition, evolutionary relationships were also found in *P. Falciparum* isolate from Papua New Guinea (AY462643.1) [19] and *P. falciparum* strain FCQ-27 from Malayan, South Africa (AF008980.1) [20].

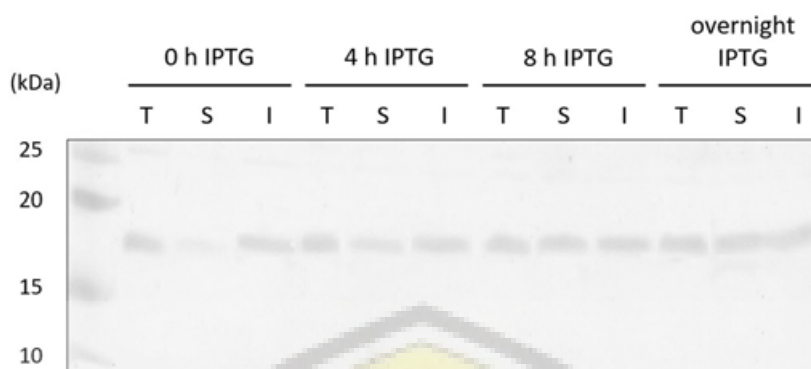


Fig. 4. The Expression of CIDR1 α -PfEMP1 recombinant protein in *E. coli* BL21(DE3). Transformed *E. coli* BL21(DE3) were grown using 0.1 mM IPTG for 0 h, four hours, eight hours, and overnight at room temperature. Total, soluble, and insoluble proteins were analyzed by Western blotting using an anti-His-6-tag antibody against epitope contained in the tag. T: total protein; S: soluble protein; I: insoluble protein.

Table 2. Residues predicted in CIDR1 α -PfEMP1 recombinant protein to be part of a B-cell epitope.

Start position	End position	Peptide sequence	Peptide length
6	21	YDDSNKNSKENNNCIQ	16
59	70	GKCINKDNGNTC	12
89	98	KNRMGQNKRT	10

It is likely that there is no clustering of CIDR1 α sequences based on geographical origin.

This study has identified the sequence characteristic of CIDR1 α -PfEMP1. We found a G nucleotide at the position of 262 which only showed homology with *P. billcollinsi*, chimpanzee-infecting *Plasmodium* sp., as described in a black rectangle in Fig. 2. The addition of a nucleotide caused translation change of nucleotide downstream of nucleotide 262. There were only 36 nucleotides (262-297) could be translated into 12 amino acids, the rest 229 nucleotides (298-526) downstream of nucleotide 297 could not be translated because there was an internal stop codon encoded by nucleotide 298-300 (TAA). Therefore, the CIDR1 α -PfEMP1 recombinant protein was expressed as a truncated protein derived from 297 nucleotides upstream which are translated into 99 amino acids.

A previous study on CIDR MC179 has predicted the structure of the domain comprised of three helices (H1, H2, H3) and three additional helices (a, b, c) joined by loops. H1 helix was connected

to H2 by a loop and H2 was linked to H3 by three additional helices consisting of ± 70 residues [10]. The amino acid sequence of our CIDR1 α domain was aligned with CIDR C1 consisting of N-terminal to H3 [7] and CIDR1 MC179 consisting of H1 to C-terminal [10]. Multiple amino acid sequence alignment showed that CIDR1 α -PfEMP1 from Indonesian *P. falciparum* isolate was composed of N-terminal, H1, a loop, and H2. Conserved cysteine areas were located on N-terminal of CIDR1 α domain, a loop between H1 and H2, and N-terminal of H2. Residues 39-40 (FF) of our sequence are the most significant conservation occupied by F655 and F656 in the CIDR1 α domain of HB3var03 PfEMP1. This conservation is essential to retain the hydrophobic nature of the protrusion, maintaining its capacity to insert into the hydrophobic groove of EPCR [21].

Malaria antigens are among the most difficult proteins to express because of their extreme genetic codon usage. *P. falciparum* genome has a very high content of adenine and thymine resulting in early termination

in the mRNA translation process. Therefore, it is only expressed as a truncated form. Surface-exposed molecules expressed by the parasite such as domains of PfEMP1 are positively charged caused by the abundance of arginine and lysine residues in their sequences thus complicating the expression in heterologous systems such as *E. coli* [22]. The rare codons in *E. coli* such as arginine, leucine, isoleucine, and proline which are frequently found in PfEMP1 (6.6-10 fold more often than in *E. coli*) will inhibit the translation process. It is probably caused by the exhaustion of tRNAs for these amino acids [22, 23].

Successful protein expression is influenced by IPTG concentration, the time point of induction, incubation time, and temperature [22-24]. CIDR1 α -PfEMP1 expression was induced with a low level of IPTG [12, 14]. Induction is initiated in log phase because the translation is highly active and the expression of the recombinant protein follows this profile [12, 22]. The expression of our recombinant protein started when induced with 0.1 mM IPTG for four hours at room temperature and more clearly expressed when induced with IPTG for eight hours at room temperature which corresponded to the previous study [7]. Our recombinant protein was expressed as a fusion protein. The advantages of heterologous protein as his-tag protein consisting of six tandem histidine residues not only include enhancing expression and solubility, but also mediating purification and protein detection by an anti-his-tag antibody in western blot analysis [25].

Solubility predictions for PfEMP1 domains in the decreasing order of their probability to be expressed in a soluble form (% mean solubility) are as follows: ATS (56.7%) > CIDR1 α (46.8%) > CIDR2 β (42.9%) > DBL2-4 γ (31.7%) > DBL2 β + C2 (30.6%) > DBL1 α (24.9%) > DBL2-7 ϵ (23.1%) > DBL2-5 δ (14.8%). The length of the domains does not correlate with their probability for successful expression in the soluble form [24]. Expression of *var gene* in *E. coli* often results in totally or partially unfolded or incorrectly folded conformation which is accumulated as aggregates and stored as inclusion bodies [22, 23]. The solubility of the protein correlates with its correct structure [22, 24]. A previous study reported that the residues in the border of N-terminal of CIDR

rC1-2 and N-terminal of H1 consisted of KEDKIMSY which were important for correct folding in *E. coli* [20]. Three out of eight amino acids (MSY) showed homology with our sequence. Proper interactions involving key residues, i.e. cysteine, also determines amino acids transformation into a correctly folded protein [24].

CIDR1 α -PfEMP1 from Indonesian isolate was predicted to have B cell epitope. Previous studies demonstrated that during parasite infection, the epitope of parasite antigen could bind to B cell receptor which will present the antigen to helper T cell and subsequently provoke B cell to produce immunoglobulin [26, 27] which is expected to generate the multiple-strains' inhibitory reaction. It is likely that the length of the sequence is related to cross-reactive stimulation. Antibodies raised against full-length MC r-179 protein had only a strain-specific inhibitory response, while antibodies against short peptides within MC r-179 induced cross-reactive inhibition effects [14]. The immunogenicity of a protein also depends on other factors such as molecular weight and amino acid composition. A protein with molecular weight over 10 kDa is an immunogenic protein. Aromatic amino acids (tyrosine, phenylalanine, tryptophan, histidine) and amino acids with positive charges (arginine, lysine, histidine) are stronger immunogens compared to the others [28]. These facts indicated that our antigenic protein has a potency to generate a specific antibody.

CONCLUSION

The CIDR1 α -PfEMP1 from Indonesian *P. falciparum* isolate had similarity to primate and human-infecting *Plasmodium spp.* isolates. The positive result of CIDR1 α amplification only from a severe malaria patient indicated its involvement in the pathogenesis of severe malaria. The CIDR1 α was predicted to have B cell epitope, implicating its potency in inducing an antibody. Further studies by *in vivo* immunogenicity test should be established for further development of its potency as a vaccine candidate.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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