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MA **muhammad ali** <maliqh@yahoo.com>
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Cc: yunitasabrina@yahoo.com


Mon, Dec 24, 2012 at 8:55 PM ☆

Dear Prof. Gholamhossein Edrissian
Editor-in-Chief, Iranian Journal Parasitology

I would like to submit the attached file of manuscript to Iranian Journal of Parasitology.
Would you please send it to reviewer of the journal.
I am looking forward the reviewer responses












Sincerely yours


Muhamad Ali, Ph.D
Laboratory of MicroBiotechnology
Faculty of Animal Sciences
Mataram University, Indonesia

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Dear Muhamad Ali

Would you please have a look at the referee(s) comments and let us know your reply to each referee separately. Besides, your corrected paper must be sent simultaneously. Your prompt reply would be of high appreciation.

Best wishes
Gh. H. Edrissian

Editor-in-Chief
Iranian J Parasitol



Iranian Society of Parasitology
Iranian Journal of Parasitology

Date: **25-12-2012**

Dear Muhamad Ali

Thank you for submitting the paper entitled:

Diversity Sequence in PfMDR1 and Conserve Sequence in pLDH of *Plasmodium falciparum* Gene from Indonesia: Its implications to Design of New Antimalarial Drugs Less Prone to Resistance

The submission number for your paper is: **P 710**
(Please mention this No. on your future contact)

Your paper will be reviewed as quickly as possible ; and we will be in touch again in due course.

Yours sincerely,
Prof. GhH Edrissian
Editor-in-Chief
Iranian J Parasitol

For queries specific to this submission please contact:
ijpa@tums.ac.ir

P.O.Box: 14155-6446, Tehran- Iran, Tel/Fax: +98-21-88953041
E-mail: ijpa@tums.ac.ir

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muhammad ali <maliqh@yahoo.com>
To: jpa@tums.ac.ir Sun, Mar 10, 2013 at 11:52 PM

Prof. Dr. ME Rokni, Ph.D
Editor
Iranian J Parasitol
Public Relations- Iranian Society of Parasitology
Tel/Fax: 021-88953041
P.O.Box: 14155-6446, Tehran- Iran

Dear Sir,
We have revised paper entitled "Sequence Diversity of *pfmdr1* and Sequence Conserve of *pldh* in *Plasmodium falciparum* from Indonesia: Its Implications on Designing a Novel Antimalarial Drug with Less Prone to Resistance" (The submission number paper is P_710) based on referee suggestion. Could you please find out the attached file of the revised paper and my answer for each referee.

Sincerely yours,
Muhamad Ali
Lab. MicroBiotechnology
Faculty of Animal Sciences, Mataram University,
Jl. Majapahat No. 62 Mataram, Indonesia

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Dear Referee A

We have revised the paper based on the referee A recommendation (point 1 to 4). However, we didn't submit the gene sequence to Genebank because the sequences are same with the previous submitted sequence (BankGene XM_001349953.1).

Sincerely yours,
Muhamad Ali

Referee A:

- 1- Write abstract in Journal format
- 2- Write references as journal format (numerical in text and collected reference on the end of paper)
- 3- There are use only five references in the text
- 4- Write discussion in separated paragraph from paper results
- 5- Submit gene in GenBank and send its accession number for Journal

Dear Referee A:

We have revised the paper based on the referee A recommendation (point 1 to 4).

Sincerely yours,
Muhamad Ali

Referee A:

- 1- Please delete the sentences from end of the introduction:
The *pfmdr1* were amplified¹⁵ and genotyped by using Restriction Fragment Length Polymorphism (RFLP)^{16,17}. Sequence analysis of *pldh* was performed using NCBI public domain database and aligned using Clustal W ver. 1.83¹⁸. Results of this research will definitely help to guide the drug policy in malaria eradication, especially in Indonesia, and as a consideration in designing new antimalarial drugs with less prone to resistance.
- 2- It is better to replace EXTRACTION by isolation (like: DNA extraction.....)
- 3- in this sentence : Since the pBluescript II KS+ vector has β -lactamase gene, LB medium containing X-gal was degraded perfectly by the *E. coli* bearing the recombinant plamid. The indicator of the degradation is white colony for recombinant plamid-bear
It could be β galactosidase (means LacZ') for blue white colony
- 4- in this sentence: cystein (S) at codon 1034 (S1034S). Cystein (C) is correct

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muhammad ali <maliqh@yahoo.com>
To: jpa@tums.ac.ir Sun, Mar 10, 2013 at 11:52 PM

Prof. Dr. MB Rokni, Ph.D
Editor
Iranian J Parasitol
Public Relations- Iranian Society of Parasitology
Tel/Fax: 021-88953041
P.O.Box: 14155-6446, Tehran- Iran

Dear Sir,
We have revised paper entitled "**Sequence Diversity of *pfmdr1* and Sequence Conserve of *pldh* in *Plasmodium falciparum* from Indonesia: Its implications on Designing a Novel Antimalarial Drug with Less Prone to Resistance**" (The submission number paper is P 710) based on referee suggestion. Could you please find out the attached file of the revised paper and my answer for each referee.

Sincerely yours,
Muhamad Ali
Lab. MicroBiotechnology
Faculty of Animal Sciences, Mataram University,
Jl. Majapahit No. 62 Mataram, Indonesia
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Dear Referee B
We have revised the paper based on the referee B suggestion. We hope this manuscript will be considered for the next processes.
Sincerely yours,
Muhamad Ali
Referee B:
The manuscript must be revised for grammatical and structure errors.
There are no results and discussion sections. References should be also inserted into text and content manuscript. After major modification manuscript will be considered.

Referee B:

- The manuscript has some incorrect words including "serious" in line 4 [page 2](#)
- 2- There are not spaces between many words in whole of manuscript
- 3-The lines 19-22 of Page 2(introduction) should be moved to materials and method section.
- 4- Information concern to prevalence of malaria in Indonesia 9 line 5 Introduction) has no reference in the text. It seems 15 million cases of clinical malaria is in valid.
- 5-Paragrapg 3 page 3 (material and Methods) is about nested PCR for diagnosis of plasmodium species and not pfmdr1
- 6-From 311 positive microscopy malaria cases only 155 cases were confirmed by nested PCR. Therefore authors should explain about dramatic difference in two method findings in discussion section.
- 7-Ten lines of page 7 (result section) should be moved to Materials and Methods section
- 8-In discussion section the findings of this study should be compare with similar studies that conducted in Indonesia and other parts of the world.

 **muhammad ali** <maliqh@yahoo.com>
To: jipa@tums.ac.ir



Prof. Dr. MB Rokni, Ph.D
Editor
Iranian J Parasitol
Public Relations- Iranian Society of Parasitology
Tel/Fax: 021-88953041
P.O.Box: 14155-6446, Tehran- Iran

Dear Sir,
We have revised paper entitled "**Sequence Diversity of *pfmdr1* and Sequence Conserve of *pldh* in *Plasmodium falciparum* from Indonesia: Its implications on Designing a Novel Antimalarial Drug with Less Prone to Resistance**" (The submission number paper is: [P 710](#)) based on referee suggestion. Could you please find out the attached file of the revised paper and my answer for each referee.

Sincerely yours,

Muhamad Ali
Lab. Microbiotechnology
Faculty of Animal Sciences, Mataram University,
Jl. Majapahit No. 62 Mataram, Indonesia

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Diversity Sequences in *pfmdr1* and Conserve Sequences in *pldh* of *Plasmodium falciparum* from Indonesia: Its Implications to Design New Antimalarial Drugs with Less Prone to Resistance

Muhammad Ali¹, Tetriswinda A Hidayatullah¹, Alimuddin², and Yunita Soetrisna³

¹Laboratory of MicroBiotechnology, Faculty of Animal Sciences, Mataram University, Mataram 83123, Indonesia ²Laboratory of Microbiology Faculty of Medical, Mataram University, Mataram, 83123, Indonesia ³Graduate School of Animal Science, Faculty of Animal Science, Mataram University, Mataram, 83123, Indonesia
*Corresponding authors: email: maliqh@yahoo.com

ABSTRACT

Background: *Plasmodium falciparum* multi-drug resistance-1 (PFMDR1) gene is a well known molecular marker for drug (chloroquine) resistance in a malaria treatment. The PFMDR1 variants are able to protect a parasite from the antimalarial drugs. Parasite lactate dehydrogenase (pLDH), a crucial enzyme for *Plasmodium* survival during their erythrocytic stages, has also been identified to be a novel target for antimalarials. Drugs directed against the enzyme would be very effective against *Plasmodium*. Therefore, conserve sequences of *pfmdr1* and *pldh* are very important for malarial eradication using chemotherapy.

Methods: *P.falciparum* genomic DNA was isolated from malaria patients in Nusa Tenggara Indonesia. *pfmdr1* was amplified using nested PCR and genotyped using Restriction Fragment Length Polymorphism (RFLP). *pldh* was amplified, sequenced, and analyzed using NCBI public domain database and alignment using Clustal W ver. 1.83.

Results: Genotyping of the *pfmdr1* showed that extremely high diversity sequences were observed among isolates. However, a sequence analysis of *pldh* indicated that open reading frame of 316 amino acids of the gene showing 100% homology to the *P. falciparum* 3D7 reference *pldh* (GeneBank: XM_001349953.1).

Conclusion: This is the first report which confirms the heterologous of *pfmdr1* and the homologous sequences of *P.falciparum pldh* isolated from Nusa Tenggara Islands of Indonesia, indicating that the chloroquine could not be used effectively as antimalarial target in the region and the pLDH-targeted antimalarial compound would have higher chance to be successful than using chloroquine for curing malaria worldwide.

Keywords: malaria, *Plasmodium falciparum*, drug resistance gene, *pfmdr1*, *pldh*, Indonesia

Introduction

Malaria, also called the "King of Diseases", remains to be the most common infectious disease and causes an enormous public health problem in tropical and subtropical regions¹⁻⁴. According to the World Malaria Report released by WHO, 247

million malaria cases were reported in 2008, and 3.3 billion people were at risky condition in 2006 from 109 countries resulting in estimated 881,000 deaths⁴. According to Indonesian health survey in 2008, 70 million people were living in endemic-malaria area (15 million cases of clinical malaria), and 56.3 million people live in mid endemic region. Recently, the malaria cases in Indonesia are concentrated in the eastern region, contributing to more than 80% of the nation's population. The latest survey conducted in 2011 showed that 45,000 cases were confirmed to be malaria⁵.

Even though intensive prevention and eradication programs were performed, malaria is still a serious health problem worldwide^{6,7}, and the emergence of *P. falciparum* resistance isolates particularly to chloroquine makes this problem even worse⁸. The resistance to the chloroquine is resulted from point mutation in multi-drug resistance-1 (PFMDR-1) gene which causes the diversity in these genes⁹⁻¹¹. Detection of molecular marker of anti-malarial drug resistance is the latest method to monitor anti-malarial drug resistance in *Plasmodium*¹².

The increasing resistance of malaria parasitic strains to conventional anti-malarial drug has stimulated the need for the development of new compounds with novel modes of action. Parasite lactate dehydrogenase (pLDH), a crucial enzyme for *Plasmodium* survival during their erythrocytic stages, has also been identified to be a novel target for antimalarials^{13,14}. Compounds that inhibit the enzyme function can represent therapeutic agents to target the disease. Therefore, study of sequence homologous of the enzyme is necessary to predict the effectiveness of the compounds.

This research was carried out to detect molecular markers of antimalarial drug resistance based on *P. falciparum* multidrug resistance 1 (*pfmdr1*) genes and the sequence of *P. falciparum* lactate dehydrogenase (*pldh*) genes. The *pfmdr1* genes were

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Fri, Jun 14, 2013 at 8:20 AM

Dear Dr MB Rokni
Editor
Iranian Journal of Parasitology

I revised my paper as suggested by reviewer. Would you please find out the revised version. I am sorry for this late because of my visiting to Toyama University-Japan for 2 month.

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• **muhammad ali** Dear Dr. MB Rokni Editor of Iranian Journal of Parasitology I attached 1st and : Mon, Jun 17, 2013 at 9:57 PM ★

• **muhammad ali** <maliqh@yahoo.com> To: ijpa@tums.ac.ir Sun, Sep 15, 2013 at 8:37 AM ★

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Best Regards

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muhammad ali <maliqh@yahoo.com> To: ijpa@tums.ac.ir Mon, Jun 17, 2013 at 9:57 PM ★

Dear Dr. MB Rokni
Editor of Iranian Journal of Parasitology

I attached 1st and 2nd (the last) revision of my paper (no 710). I also attached my reply for each referee.
Thank you very much for your cooperation.

Best regards

Dr. Muhamad Ali
Laboratory of MicroBiotechnology Mataram University

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Dear muhammad ali

Thank you for your cooperation with this journal.your paper is sent for edit and will be published in this journal.

Best Regards

IJP office

On Sun, Sep 15, 2013 at 5:07 AM, muhammad ali <maliqh@yahoo.com> wrote:

Dear Dr MB Rokni

I would like to know the last information about my paper (P 710) that have I revised 2 times based on referee correction. Hopefully I can get the information soon.

Best Regards

Muhamad Ali

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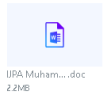
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Editor- Iranian J Parasitol
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Muhamad Ali
Lab. Microbiology and Biotechnology
Mataram University, Indonesia
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Dear colleague

Would you please have a look at the comments in the text and correct the paper accordingly.

- ✓ Because some modifications have been made on your paper, please revise the present issue only not your original one.
- ✓ Please do not delete any comments of the text.

Please pay attention that due to large papers presented here, if we do not receive any reply within **3 days**, the paper will be omitted of the circulation.

Best regard

Dr MB Rokni
Editor

Original Article

Sequence Diversity of *pfmdr1* and Sequence Conserve of *pldh* in *Plasmodium falciparum* from Indonesia: Its implications on Designing a Novel Antimalarial Drug with Less Prone to Resistance

**Zorica TERZIC SUPIC¹, Milena SANTRIC MILICEVIC¹, Isidora SBUTEGA¹, Vladimir VASIC²*

¹ Institute of Social Medicine, Medical Faculty, University of Belgrade, Belgrade, Serbia

² Institute of Dermatology, Clinical Center of Serbia, Belgrade, Serbia

³ Dept. of Statistics and Mathematics, Faculty of Economics, University of Belgrade, Belgrade, Serbia

Muhamad Ali^{1*}, Tetrawindu A Hidayatullah², Almuddin³, Yunita Sabrina²

Date:2013-09-21
No.: 541



Iranian Society of Parasitology

Iranian Journal of Parasitology

Dear Muhamad Ali


Here by it is my pleasure to announce that your manuscript submitted to the **Iranian J Parasitol**, entitled:

Diversity Sequence in PfMDR1 and Conserve Sequence in pLDH of *Plasmodium falciparum* Gene from Indonesia: Its implications to Design of New Antimalarial Drugs Less Prone to Resistance

has been accepted for printing after exact peer review process. The paper will be released in our next issue of the journal after amending the galley proof on your side.

Once again I do appreciate your collaboration with this journal and wish to receive your invaluable manuscripts in near future.

Best wishes


Prof. Gh. H. Edrissian

Editor-in-Chief
Iranian J Parasitol

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