

VOLUME 24 • NUMBER 9

SEPTEMBER 2018

www.aspbs.com/science

Advanced

SCIENCE

A Journal Dedicated to All Aspects
of Scientific Research

LETTERS

Editor-in-Chief: Dr. Hari Singh Nalwa, USA

Special Sections on

2017 International Conference on Actual Economy: European Discourse on Global Challenges
Paris, 27–29 November, 2017

GUEST EDITOR: Denis Ushakov

2nd International Conference on Global Health (ICGH)
Indonesia, 14–16 August, 2017

GUEST EDITORS: Budi Wiweko, Hariyono Winarto, Erlina Burhan, Indah Suci Widyahening,
Rina Agustina, F. J. Shanty Olivia, Bambang Widyantoro, Chyntia Olivia Maurine Jasirwan,
Justin Chu, and Jan (Leendert) Nouwen



AMERICAN
SCIENTIFIC
PUBLISHERS

Advanced Science Letters

ISSN: 1936-6612 (Print); EISSN: 1936-7317 (Online)
Copyright © 2000- 2018 American Scientific Publishers. All Rights Reserved.

CONTENTS

Destination Branding as a Tool for Sustainable Tourism Development (The Case of Bangkok, Thailand)

pp. 6339-6342(4)

Authors: *Van, Hung Tran; Huu, Ai Tran; Ushakov, Denis*

The Strategy of Thai Medical Services Promotion at Russian Markets

pp. 6343-6346(4)

Authors: *Pavlenko, Irina; Polishchuk, Elena; Pilyavskiy, Valery; Ushakov, Denis*

Environmental Taxes as a Tool for Environmental Protection

pp. 6347-6349(3)

Author: *Válek, Juraj*

Management of Water Companies at the Regional Level in Slovakia

pp. 6350-6352(3)

Authors: *Janas, Karol; Kucharčík, Rudolf*

Selected Peer-Reviewed Articles from the 2nd International Conference on Global Health (ICGH), Indonesia, 14–16 August, 2017

pp. 6353-6356(4)

Authors: *Wiweko, Budi; Winarto, Hariyono; Burhan, Erlina; Widyahening, Indah Suci; Agustina, Rina; Olivia, F. J. Shanty; Widyantoro, Bambang; Jasirwan, Chyntia Olivia Maurine; Chu, Justin; Nouwen, Jan (Leendert)*

A Comparison of Structural Changes in Brain MRI Between Controlled and Uncontrolled Hypertensive Patients: A Preliminary Study

pp. 6357-6360(4)

Authors: *Penduff, Yann; Ramli, Yetty; Liem, Isabella Kurnia*

A Scoring System for Preeclampsia Screening Based on Maternal and Biophysical Factors: Result from a 3-Month Cohort Study in Jakarta, Indonesia

pp. 6361-6365(5)

Authors: *Syahaarutsa, Danny Maesadatu; Purwosunu, Yuditiya*

Activity of Fractions from *Garcinia latissima* Miq. Leaves Ethyl Acetate Extract as Antibacterial Against *Bacillus subtilis* and Antioxidant

pp. 6366-6370(5)

Authors: *Ambarwati, Neneng Siti Silfi; Elya, Berna; Sa'adah, Aisyah Nur; Puspitasari, Nuraini; Malik, Amarila; Hanafi, Muhammad*

Adolescent Compliance on Iron Tablet Consumption: A Systematic Review

pp. 6371-6375(5)

Authors: *Apriani, Mirza; Syafiq, Ahmad*

Annexin A10 Expression in Hyperplastic Polyp, Sessile Serrated Adenoma Polyp and Conventional Adenoma

pp. 6376-6380(5)

Authors: *Farida, Falaivi; Rini, Handjari Diah; Nur, Rahadiani*

Antihypertensive Drug Interaction Analysis in Outpatient Patients at Pasar Minggu Regional Public Hospital in Period of July–December 2016

pp. 6381-6383(3)

Authors: *Puspitasari, Atika Wahyu; Azizahwati; Nada, Ifani Pinto*

Antioxidant Activity Test of Fraction from Ethyl Acetate Extract of *Garcinia Bancana* Miq. Leaves by DPPH and FRAP Methods

pp. 6384-6387(4)

Authors: *Azizah, Putrie Fiana; Elya, Berna; Katrin*

Antiviral Activity of *Cassia Alata* and *Cosmos Caudatus* Leaf Extract to Dengue Virus Serotype-2 New Guinea C Strain in Huh7-it1 Cell

pp. 6388-6392(5)

Authors: *Prasetya, Septian Ika; Tamujaya, Adryan; Ratnasari, Shierly; Desti, Hidayati; Louisa, Melva; Angelina, Marisha; Sudiro, Tjahjani Mirawati; Dewi, Beti Ernawati*

Association Between Food Insecurity Status Towards Low Birth Weight Among 0–11-Month-Old Infants in Sambas District, West Kalimantan

pp. 6393-6397(5)

Authors: *Jonatan, G. S; Hasanah, N; Kusuma, S; Pramesthi, I. L; Ananda, A. J. N; Ermayani, E*

Association Between Mortality and Vasoactive Agent Initiation Time During Fluid Resuscitation in Septic Shock

pp. 6398-6401(4)

Authors: *Aristya, Lara; Aditiansih, Dita*

Association of Stroke Risk Factors and Asymptomatic Intracranial Arterial Stenosis Assessed by Transcranial Doppler in Stroke Prone Person in RSUP Dr. Mohammad Hoesin Palembang

pp. 6402-6405(4)

Authors: *Junaidi, A; Marisdina, S; Masita; Indrajaya, T; Bahar, E*

Awareness of Diabetic Eye Disease and the Source of Information in a Semi-Urban Population in East Nusa Tenggara, Indonesia

pp. 6406-6408(3)

Author: *Permatasari, Dita*

Baseline Parasite Profile in Developing Irradiation Malaria Vaccine in Indonesia:
Molecular Analysis of Papua Samples

pp. 6409-6413(5)

Authors: Syaifudin, Mukh; Darlina; Nurhayati, Siti; Rahardjo, Tur; ES, Harry Nugroho; Asih, Puji Budi S; Marantina, Sylvia S; Syafruddin, Din; Yuliwati; Octavian, Antonius; Anwar, d Lidwina

Behavior and Perception of HIV Transmission Among Gay Men in Bandar Lampung; A
Qualitative Study

pp. 6414-6415(2)

Author: Larasati, T. A

Changes in Specific Activity of Glutamate Pyruvate Transaminase Enzyme and Glucose
Liver Tissue Rat (*Rattus norvegicus*) Post-Termination of Use of MSG Above Dosage
Recommendations

pp. 6416-6420(5)

Authors: Munir, M. Misbakhul; Prijanti, Ani Retno; Mudjihartini, Ninik; Ridwan, Rahmawati; Aulia, J. Ahmad

Advanced Science Letters

ISSN: 1936-6612 (Print); EISSN: 1936-7317 (Online)
Copyright © 2000- 2018 American Scientific Publishers. All Rights Reserved.

CONTENTS

Destination Branding as a Tool for Sustainable Tourism Development (The Case of Bangkok, Thailand)

pp. 6339-6342(4)

Authors: *Van, Hung Tran; Huu, Ai Tran; Ushakov, Denis*

The Strategy of Thai Medical Services Promotion at Russian Markets

pp. 6343-6346(4)

Authors: *Pavlenko, Irina; Polishchuk, Elena; Pilyavskiy, Valery; Ushakov, Denis*

Environmental Taxes as a Tool for Environmental Protection

pp. 6347-6349(3)

Author: *Válek, Juraj*

11

Advanced Science Letters

ISSN: 1936-6612 (Print); EISSN: 1936-7317 (Online)
Copyright © 2000- 2018 American Scientific Publishers. All Rights Reserved.

EDITORIAL BOARD

EDITOR-IN-CHIEF

Dr. Hari Singh Nalwa, USA

Editorial Office:

ADVANCED SCIENCE LETTERS

American Scientific Publishers
26650 The Old Road, Suite 208
Valencia, California 91381-0751, USA
Tel. (661) 799-7200
Fax: (661) 799-7230
E-mail: science@aspbs.com

ASIAN EDITOR

Dr. Katsuhiko Ariga, PhD
Advanced Materials Laboratory
National Institute for Materials Science
1-1 Namiki, Tsukuba, Ibaraki 305-0044, JAPAN

ASSOCIATE EDITORS

Diederik Aerts (Quantum theory, Cognition, Evolution theory)
Brussels Free University, Belgium.

Yakir Aharonov (Physics, Quantum Physics)
School of Physics and Astronomy, Israel.

Peter C. Aichelburg (Gravitation)
University of Vienna, Austria.

Jim Al-Khalili (Foundations of Physics, Nuclear Reaction Theory)
University of Surrey, UK.

Jake Blanchard (Engineering Physics, Nuclear Engineering)
University of Wisconsin–Madison, USA.

Simon Baron-Cohen (Cognitive Neuroscience)
University of Cambridge, UK.

Franz X. Bogner (Cognitive Achievement)
University of Bayreuth, Germany.

John Borneman (Anthropology)
Princeton University, USA.

John Casti (Complexity Science)
Internationales Institut für Angewandte Systemanalyse, Austria.

Masud Chaichian (High Energy Physics, String Theory)
University of Helsinki, Finland.

Sergey V. Chervon(Gravitation, Cosmology, Astrophysics)
Ulyanovsk State Pedagogical University, Russia

Kevin Davey (Philosophy of Science)
University of Chicago, Chicago, USA.

Tania Dey (Colloids/Polymers/Nanohybrids)
Canada.

Roland Eils (Bioinformatics)
Deutsches Krebsforschungszentrum Heidelberg, Germany.

Thomas Görnitz (Quantum theory, Cosmology)
University of Frankfurt, Germany.

Bert Gordijn (Nanoethics, Neuroethics, Bioethics)
Radboud University Nijmegen, The Netherlands.

Ji-Huan He (Textile Engineering, Functional Materials)
Soochow University, Suzhou, China.

Nongyue He (Biosensors/Biomaterials)
China.

Irving P. Herman (Materials and Solid State Physics)
Columbia University, USA.

Dipankar Home (Foundations of Quantum Mechanics)
Bose Institute, Kolkata, India.

Jucundus Jacobeit (Climate, Global Change Ecology)
University of Augsburg, Germany.

Yuriy A. Knirel (Bioorganic Chemistry)
N. D. Zelinsky Institute of Organic Chemistry, Russia.

Arthur Konnerth (Neurophysiology, Molecular Mechanisms)
University of Munich, Germany.

G. A. Kourouklis (Physics Solid State Physics)
Aristotle University Thessaloniki, Greece.

Peter Krammer (Genetics)
Deutsches Krebsforschungszentrum Heidelberg, Germany.

Andrew F. Laine (Biomedical Engineering)
Columbia University, USA.

Minbo Lan (Organic Functional Materials)
China.

Martha Lux-Steiner (Physics, Materials Science)
Hahn-Meitner-Institut Berlin, Germany.

Klaus Mainzer (Complex Systems, Computational Mind, Philosophy of Science)
University of Augsburg, Germany.

JoAnn E. Manson (Medicine, Cardiovascular Disease)
Harvard University, USA.

Mark P. Mattson (Neuroscience)
National Institute on Aging, Baltimore, USA.

Lucio Mayer (Astrophysics, Cosmology)
ETH Zürich, Switzerland.

Karl Menten (Radioastronomy)
Max-Planck-Institut für Radioastronomie, Germany.

Yoshiko Miura (Biomaterials/Biosensors)
Japan.

Fred M. Mueller (Solid State Physics)
Los Alamos National Laboratory, USA.

Garth Nicolson (Illness Research, Cancer Cell Biology)
The Institute for Molecular Medicine, Huntington Beach, USA.

Nina Papavasiliou (DNA Mutators, Microbial Virulence, Antiviral Defence, Adaptive Immunity, Surface Receptor Variation)
The Rockefeller University, New York, USA.

Panos Photinos (Physics)
Southern Oregon University, USA.

Zhiyong Qian (Biomedical Engineering, Biomaterials, Drug Delivery)
Sichuan University, CHINA.

Reinhard Schlickeiser (Astrophysics, Plasma Theory and Space Science)
Ruhr-Universität Bochum, Germany.

Surinder Singh (Sensors/Nanotechnology)
USA.

Suprakas Sinha Ray (Composites/Polymer Science)
South Africa.

Koen Steemers (Architecture, Environmental Building Performance)
University of Cambridge, UK.

Shinsuke Tanabe (Environmental Chemistry and Ecotoxicology)
Ehime University, Japan.

James R. Thompson (Solid State Physics)

The University of Tennessee, USA.

Uwe Ulbrich (Climat, Meteorology)
Freie Universität Berlin, Germany.

Ahmad Umar (Advanced Materials)
Najran University, Saudi Arabia.

Frans de Waal (Animal Behavior and Cognition)
Emory University, USA.

EDITORIAL BOARD

Filippo Aureli, Liverpool John Moores University, UK

Marcel Ausloos, Université de Liège, Belgium

Martin Bojowald, Pennsylvania State University, USA

Sougato Bose, University College, London, UK

Jacopo Buongiorno, MIT, USA

Paul Cordopatis, University of Patras, Greece

Maria Luisa Dalla Chiara, University of Firenze, Italy

Dionysios Demetriou Dionysiou, University of Cincinnati, USA

Simon Eidelman, Budker Institute of Nuclear Physics, Russia

Norbert Frischauf, QASAR Technologies, Vienna, Austria

Toshi Futamase, Tohoku University, Japan

Leonid Gavrilov, University of Chicago, USA

Vincent G. Harris, Northeastern University, USA

Mae-Wan Ho, Open University, UK

Keith Hutchison, University of Melbourne, Australia

David Jishiashvili, Georgian Technical University, Georgia

George Khushf, University of South Carolina, USA

Sergei Kulik, M.V.Lomonosov Moscow State University, Russia

Harald Kunstmann, Institute for Meteorology and Climate Research, Forschungszentrum Karlsruhe, Germany

Alexander Lebedev, Laboratory of Semiconductor Devices Physics, Russia

James Lindesay, Howard University, USA

Michael Lipkind, Kimron Veterinary Institute, Israel

Nigel Mason, Open University, UK

Johnjoe McFadden, University of Surrey, UK

B. S. Murty, Indian Institute of Technology Madras, Chennai, India

Heiko Paeth, Geographisches Institut der Universität Würzburg, Germany

Matteo Paris, Università di Milano, Italia

David Posoda, University of Vigo, Spain

Paddy H. Regan, University of Surrey, UK

Leonidas Resvanis, University of Athens, Greece

Wolfgang Rhode, University of Dortmund, Germany

Derek C. Richardson, University of Maryland, USA

Carlos Romero, Universidade Federal da Paraíba, Brazil

Andrea Sella, University College London, London, UK

P. Shankar, Indira Gandhi Centre for Atomic Research, Kalpakkam, India

Surya Singh, Imperial College London, UK

Leonidas Sotiropoulos, University of Patras, Greece

Roger Strand, University of Bergen, Norway

Karl Svozil, Technische Universität Wien, Austria

Kit Tan, University of Copenhagen, Denmark

Roland Triay, Centre de Physique Théorique, CNRS, Marseille, France

Rami Vainio, University of Helsinki, Finland



Baseline Parasite Profile in Developing Irradiation Malaria Vaccine in Indonesia: Molecular Analysis of Papua Samples

Mukh Syaifudin^{1,*}, Darlina¹, Siti Nurhayati¹, Tur Rahardjo¹, Harry Nugroho ES¹, Puji Budi S. Asih², Sylvia S. Marantina², Din Syafruddin², Yuliwati³, Antonius Octavian⁴, and Lidwina Anwar⁴

¹Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency, Jakarta, Indonesia

²Malaria Group, Eijkman Institute for Molecular Biology, Jakarta, Indonesia

³Jayapura General Hospital, Papua, Indonesia

⁴Biomedicine Center, National Institute of Health Research and Development, Ministry of Health, Papua, Indonesia

Introduction: Malaria is still a major problem in Indonesia. Therefore the development of its vaccine that can be created with radiation is urgently needed. This research aims to determine the genotype of blood specimens obtained from 4–22 years old malaria outpatients in Jayapura General Hospital, Papua. The information obtained will be a great value in additional knowledge for vaccine development of irradiated gamma-ray parasites. **Methodology:** Microscopic examination on thin blood smear was done, followed by Polymerase Chain Reaction (PCR) diagnosis to further confirm the parasite using 18S rRNA gene and mitochondrial primers on deoxyribonucleic acid extracted using chelex-100 ion exchanger and the presence of each mutation in the genes was examined using restriction fragment length polymorphism method. **Results:** Our result showed that nine samples infected with *Plasmodium falciparum* and 1 with *P. vivax*. Beside that nine samples carried mutations in *pfprt* and 22% in *pfmdr1*. Mutations involving N1042D in *pfmdr1* gene was also detected in 3 samples (33%). Mutations in *dhr* gene at codon S108N were detected in all nine samples. Mutations in this gene at codon K540E have also detected in 3 (33%) samples. **Conclusion:** Analysis of the parasite genotype indicated that infection occurred with multiple parasite strains. The majority of the samples carried the 3D7 type of MSP2 gene while others carried the FC27 type. The MSP1 genes observed carried K1, MAD20, and RO33 types but some other types could not be determined.

Keywords: Genotyping, Malaria, Microscopic, PCR, RFL.

1. INTRODUCTION

Malaria continues to be one of the foremost causes of death in the developing countries such as Indonesia. In this country, around 15 million cases and 42,000 deaths were found every year.^{1–3} The anti-malarial drug only partially effective in controlling disease due to the increasing problem of drug-resistant parasites all over the world. Thus, additional approaches such as vaccination have become a top public health priority for malaria control.⁴

Throughout Papua, Indonesia, malaria has been an important disease for many years, accounting for 16% of all hospitalizations, 14% of all hospital deaths, and 20% of all outpatient consultations.^{5,6} Consequently, most febrile patients are considered to have malaria and are given empirical treatment out of fear of missing life-threatening *P. falciparum* infection.⁷ Several factors may promote the development of antibiotic and anti-malarial resistance and unnecessary morbidity and mortality. The

first chloroquine-resistant isolates of *P. vivax* were reported from Papua in 1989.^{8,9} This region currently has the highest rates of drug-resistant *P. vivax* in the world.¹⁰

Ionizing radiation has been successfully used to attenuate parasites and micro-organisms for vaccine development and research.^{11–14} Irradiated pathogens frequently lost their reproductive ability or virulence, but retain the metabolic activities and morphology, and can stimulate a specific immune response. In some cases, the radio-attenuated pathogens are more immunogenic than the normal counterparts.¹⁵

Several *P. falciparum* genes show extensive genetic polymorphism. This phenomenon is exploited for genetic fingerprinting and for assessing parasite population dynamics. High polymorphism has been shown in *msh1*, *msh2* and *glurp* genes in different geographical locations in malaria endemic areas.^{16–21} Therefore, comparing the genotypes of these three loci at baseline and at the time of parasite recurrence would be expected to discriminate between recrudescence and new infections.^{22,23} Comparing

*Author to whom correspondence should be addressed.

molecular genotypic pattern of pre-treatment (baseline) and recurrent infections provides a means to help characterize the recurrent parasites as a recrudescence, i.e., a true failure, or a new infection (either from pre-existing liver infection or a newly established infection from an infected mosquito bite), i.e., a successful treatment.

It is already known that a malaria vaccine would be a valuable tool that offers the potential to reduce both the morbidity and mortality of the disease. The parasite has a huge repertoire of possible antigenic variants, this together with the ability to undergo rapid antigenic switching presents an important challenge to vaccine development. It has been over 30 years since the first successful malaria vaccine was trialed.²⁴ As it consisted of the bites of a thousand irradiated mosquitoes infected with the parasite. The feasibility of this approach option for large-scale use has been until recently dismissed. Further elucidation of the complex geographical patterns of *P. vivax* and *P. falciparum* variation will be important both for diversity assessments of genes encoding candidate vaccine antigens and in the formulation of control and surveillance measures aimed at malaria elimination.

Our results confirmed the propriety of this system for large-scale genetic analysis of *P. falciparum* which will provide innovative insight into the complex genetic structure of the malaria parasite and hugely accelerate efforts to develop novel intervention strategies such as a vaccine that can be created from attenuated parasites with ionizing radiation.

2. METHODS

2.1. Subjects

This study is part of an experimental research on the development of irradiated sporozoites as malaria vaccine materials that took place in Jayapura, Papua Province, Indonesia with quite highly malaria transmission intensities. For all the studies ethical approval was obtained from Ethics Committees of National Institute of Health Research and Development, Ministry of Health. Informed consent form was signed by all subjects who suspected infected with malaria parasites as they were as outpatient in Jayapura General Hospital. And blood samples from participant were collected in 5.0 ml BD Vacutainer. In this research, 10 blood samples were studied.

2.2. Microscopic Examinations

Thick blood smears were prepared for all blood samples and stained with 10% Giemsa. The malaria parasite was detected under light microscopy using 1,000 \times oil immersion by an experienced microscopists from the Hospital. At least 200 ocular fields were examined before considering a slide negative, and parasite densities were counted as parasites per 200 leukocytes and reported as parasites/mm³ assuming a white blood cell count of 8,000/mm³.²⁵

2.3. DNA Extraction

Parasite and human host DNA was obtained from blood samples using chelex-100 ion exchange (Biorad Laboratories, Hercules, CA, USA) according to a procedure described previously.²⁶ The DNA was either used immediately for PCR amplification or stored at -20 °C for later analysis.

2.4. Molecular Genotyping

PCR amplification of template DNA and analysis of region II of *glurp*, central polymorphic region of *msp2* (3D7 and FC27 allelic families), and block 2 of *msp1* (K1, MAD20, and RO33 allelic families) was performed in accordance to the recently recommended genotyping protocol²⁷ with minor modification. The primary and nested PCR amplification of the *msp1* and *glurp* loci were carried out at Malaria Laboratory of Eijkman Institute, and the conditions were as described elsewhere.²⁸ The DNA amplified was analyzed by electrophoresis on 2% agarose gel and stained in 0.5 μ g/ml of ethidium bromide solution, and the band was visualized under UV light.

2.5. Molecular Resistance Examination

To identify the presence of point mutations in molecular markers of *P. falciparum* drug resistance, we performed a series of PCRs with specific primers that amplify informative regions of the *P. falciparum* multidrug resistance 1 (*pfmdr1*), *P. falciparum* chloroquine (CQ) resistance transporter (*pfcr1*), and dihydrofolate reductase (*dhfr*) genes. Part of the *pfcr1* gene containing the first predicted transmembrane domain was amplified as described.^{13,28} The PCR products were analyzed for the Lys76Thr mutation by restriction fragment length polymorphism using *ApoI* (New England Biolabs, Beverly, MA). Restricted amplification products were subjected to electrophoresis on 10% polyacrylamide gels and visualized by staining with ethidium bromide. The regions of the *dhfr* and *dhps* gene containing the mutations involved in anti folate resistance were also amplified as described.²⁸ Mutations in the *dhfr* gene at codon Ser108Thr/Asn were identified with mutation-specific primers. Mutations in the *dhfr* gene at codons Ala16Val, Asn51Ile, Cys59Arg, and Ile164Leu and in the *dhps* gene at codons Ser436Phe, Ala437Gly, Lys540Glu, Ala581Gly, and Ala613Ser were also determined by a nested PCR amplification followed by size fractionation on agarose gels.²⁸

3. RESULT

3.1. Microscopic Observation

Microscopic and molecular examinations followed by restriction test were subjected to all samples according to the standard procedures. Results showed that microscopically all blood smears were infected with malaria parasites, of which one was infected with *P. vivax* (Fig. 1). This was confirmed by polymerase chain reaction (PCR) that revealed that nine subjects had *P. falciparum* infection and one was *P. vivax* malaria.

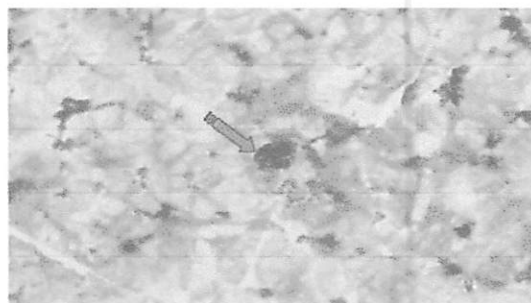


Fig. 1. Giemsa stained thick blood smear showing *P. vivax* parasite infection in one sample found in this research (arrow).

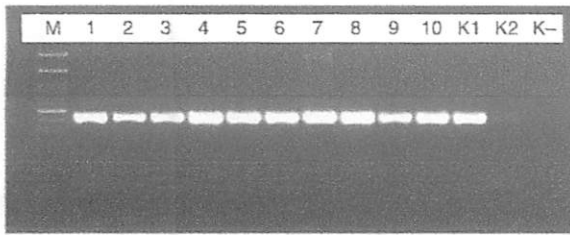


Fig. 2. PCR results in DNA mitochondrial gene where all are positive of *Plasmodium sp.*

3.2. Species Identification

PCR analysis using mitochondrial gene found that all samples were positive for *Plasmodium sp* (Fig. 2). However, genotyping using *Glurp* primer revealed that one sample was negative (Fig. 3). Identification of species with restriction fragment length polymorphism (RFLP) using *Ssp1* enzyme showed that nine were positive for Pf and 1 sample was *P. vivax* infection, whereas with *Acl1* enzyme 1 sample was Pv and nine samples were Pf. A total of 9 *msp1* genotypes (three MAD20-, two RO33- and four K1-types); 9 *msp2* genotypes (7 3D7 and 2 FC27-types) and nine *glurp* genotypes were recorded (Table I). From these, it can be known that all samples were positive for malaria with mostly were *P. falciparum* infections and the development of vaccine should be focused on this species. *Msp-1* and *msp-2* are highly polymorphic single copy genes and have been employed to investigate parasite genetic diversity all around the world, and also are under extreme immune selection pressure.

Thick-section microscopy of blood-stage *P. vivax* parasites infected sample from study area is presented in Figure 3. There is no trophozoite, ring and schizont parasites forms are shown in this figure. However, it was recognized based on its specific characteristics of thick blood smear.

3.3. SNP Study

Further study in the 16 single nucleotide polymorphisms covering *pfprt*, *Pfmdr1*, *dhfr* and *dhps* genes on these ten samples revealed that two out of 9 samples analyzed had a polymorphism. The complete results are presented in Table II. These are found in sample no. 7 and eight where polymorphism is N86Y.

Here *AflIII* restriction enzyme was used to analyze and detect mutation point at codon 86 (N86Y) and *DdeI* for mutation point at codon 1034 (S1034). Amplification of *pfmdr1* generated

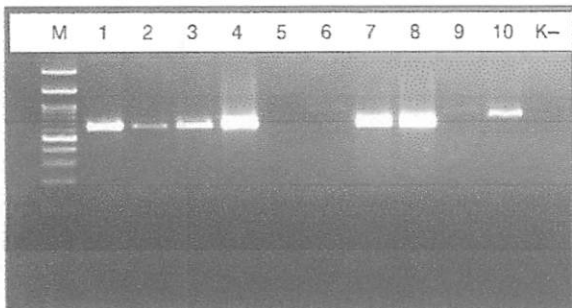


Fig. 3. Genotyping of the samples using *Glurp* primers where one sample was negative (sample no. 5).

Table I. Positive results in MSP1, MSP2, and *Glurp* from 10 samples analyzed.

No. sample	MSP1			MSP2		<i>Glurp</i>
	MAD 20	K1	RO33	3D7	FC27	
1	-	+	-	+	-	+
2	-	-	-	+	-	+
3	-	+	-	+	-	+
4	-	+	-	+	-	+
5	-	-	-	-	-	-
6	+	-	-	-	+	+
7	-	-	+	+	-	+
8	-	-	+	-	-	+
9	+	+	-	+	+	+
10	+	-	-	+	-	+

372-bp PCR product. Restriction of the fragment using *AflIII* produced 248-bp and 124-bp in the mutation of N to Y at position 86. Whereas, the amplification of *pfmdr1* using 1034-F and 1034-R (Table I) generated 189-bp PCR product (Table II). Three polymorphisms were lack to be analyzed due to the fail in enzymatic cleavage that was due to an appropriate condition of PCR and enzymatic process.

In this research nine samples carried mutations in *pfprt* (all sample showed nucleotide alteration from K to T) and 5 of 9 (22%) sample had mutation in *pfmdr1* (2 of them were in N86Y). Mutations involving N1042D in *pfmdr1* gene was also detected in 3 samples (33%). Mutations in this gene at codon K540E were also detected in 3 (33%) samples (no. 6, 7 and 8). No results were obtained for S1034C mutation of *Pfmdr-1* and A16V of *dhfr* genes as well as in *Atp-6* due to technical problem.

More studies are warranted to examine the same thing with much larger number of samples to get a more comprehensive pattern in genotyping the parasites for development of irradiation vaccine for protective immunity.

Table II. Detailed SNP results of each genes representing their nucleotide alterations on ten samples of parasited infected blood obtained from Papua, Indonesia. Ten samples were studied for 15 SNPs of *P. falciparum*.

Poly-morphism	No. of samples and its nucleotide alterations									
	1	2	3	4	5	6	7	8	9	10
<i>Pfprt</i> gene										
K76T	T	T	T	T	-	T	T	T	T	T
<i>Pfmdr-1</i> gene										
N86Y	N	N	N	N	-	N	Y	Y	N	N
S1034C	ND	ND	ND	ND	-	ND	ND	ND	ND	ND
N1042D	N	N	N	N	-	N	D	D	D	N
D1246Y	D	D	D	D	-	D	D	D	D	D
<i>dhfr</i> gene										
S108N	N	N	N	N	-	N	N	N	N	N
N51I	N	N	N	N	-	N	N	N	N	N
C59R	C	C	C	C	-	C	C	C	C	C
A16V	ND	ND	ND	ND	-	ND	ND	ND	ND	ND
<i>dhps</i> gene										
A437G	A	A	A	A	-	A	A	A	A	A
K540E	K	K	K	K	-	E	E	E	K	K
S436A	S	S	S	S	-	S	S	S	S	S
G581A	G	G	G	G	-	G	G	G	G	G
A581G	G	G	G	G	-	G	G	G	G	G
A613S/T	S/T	S/T	S/T	S/T	-	S/T	S/T	S/T	S/T	S/T
Atp-6	ND	ND	ND	ND	-	ND	ND	ND	ND	ND

Notes: Sample no. 5 as *P. vivax* infected blood was not determined, ND: no data available due to the lack of enzymatic cleavage.

4. DISCUSSION

Malaria poses a serious public health problem in many parts of the world and approximately half of the world's population is at risk, in particular those living in lower-income countries.²⁹ According to US Global Health Policy Reported Malaria Deaths 2010, Indonesia ranks 28 of countries with highest case of malaria (432 cases/100,000).³⁰ It is by the fact that malaria remains a major public health problem in Indonesia, with 30 million cases and 120,000 deaths annually. Recently measures of annual parasite incidence (API) have varied substantially between provinces, but the highest API is consistently detected in the eastern parts of Indonesia. Moreover, the control of malaria in Indonesia is still problematic due to lack of access to health facilities in very remote areas and hard to reach islands, inequality of health development, and limitation of adequate malaria human resources mostly microscopist and entomologist as well as genotypic.³¹

Antibodies to polymorphic antigens expressed during the parasites at erythrocytic stages are important mediators of protective immunity against *P. falciparum* malaria. Therefore, polymorphic blood stage antigens such as MSP3, EBA-175 and GLURP and variant surface antigens PfEMP1 and PfPR are considered vaccine candidates. Molecular genotyping of highly polymorphic regions of *P. falciparum* msp1, msp2 and glurp loci is usually carried out to distinguish recrudescence (true failures) from new infections.^{32,33} This tool has now been adopted as an integral part of anti-malarial and vaccine efficacy studies and clinical trials. However, there are concerns over its utility and reliability because conclusions drawn from molecular typing depend on the genetic profile of the respective parasite populations, but this profile is not systematically documented in most endemic areas of Indonesia to support the development of malaria vaccine with irradiation technique.^{5,34}

We describe an analysis of the genetic variation of malaria parasites in a very limited number of samples compared to the high number of incidence that commonly found in Jayapura. Large-scale number should be conducted to maximise the statistical power and to avoid a little information on the development of vaccine for this disease created by ionizing radiation. Here we selected two polymorphic markers, the genes for merozoite surface protein 1 (MSP1) and MSP2, to genotype the *P. falciparum* isolates responsible for the infection. PCR amplification of these genes points out the presence of length polymorphism, allowing the detection of multiple infections by different *P. falciparum* genotypes.³⁵

Genotype diversity existed among *P. falciparum* parasite in several countries such Colombia, China and Suriname. Villa et al.³⁶ from Colombia found wild-type form in codons 16, 59 and 164 of *dhfr* gene, while mutant found in codons 51 and 108. In *dhps* gene, the mutant 437 glycine was detected in 85% sample, while codons 436, 540, 581 and 613 were wild-type. While a study in China by Huang et al.³⁷ on total of 108 blood samples found that almost all (95.3%) parasites still carried the *pfprt* K76T mutation, whereas the majority of isolates displayed the wild-type pfmdr1 N86 and D1246 sequences. The most prevalent mutation was pfdhfr C59R (95.9%). A437G ($n = 77$) and K540E ($n = 71$) were the most prevalent mutations in pfdhps, and 52.7% of the samples were double mutants, among which A437G/K540E was the most common double mutation (37/49). Quadruple mutants were found in 28.0% (26/93) of

the samples. A total of 8.6% of isolates (8/93) carried the S436A/A437G/A581G triple mutation. Other study in Suriname on 86 patients with symptomatic malaria had been done. In this country mutations in the *pfprt*, *dhps*, and *dhfr* genes were found in all samples tested, suggesting that resistance to chloroquine and antifolate drugs is present at a high frequency. A low number of alleles was found for the *msp-2* and *glurp* genes.³⁸

5. CONCLUSION

In conclusion, our analysis showed that mostly blood samples were infected with *P. falciparum*. The majority of the samples carried the 3D7 type of MSP2 gene while others carried the FC27 type. The MSP1 genes observed carried K1, MAD20 and RO33 types but some other types could not be determined.

Acknowledgments: We would like to thank the Director and all staffs of Jayapura General Hospital who permitting the sample collection. We also acknowledge the National Nuclear Energy Agency of Indonesia for supporting the research grant of 2014 budget.

References and Notes

- SEARO, Malaria situation in SEAR countries: Indonesia, http://www.searo.who.int/en/Section10/Section21/Section340_4022.htm (2009).
- UNDP, Indonesia progress report on the millennium development goals 2004, Tech. Rep., United Nation Development Program Jakarta, Indonesia (2004).
- Ministry of Health, RI-CDC, Malaria Situation in Indonesia—unpublished report, Tech. Rep., Ministry of Health-Directorate of Communicable Disease Control, Jakarta, Indonesia (2006).
- World Health Organization, The Initiative for Vaccine Research: Strategic Plan 2006–2009, World Health Organization, Geneva (2006).
- N. H. Punjabi, W. R. Taylor, G. S. Murphy, S. Punwaningsih, H. Picarima, J. Sisson, J. G. Olson, S. Baso, F. Wangsaputra, M. Lesmana, B. A. Oyofa, C. H. Simanjuntak, D. Subekti, A. L. Corwin, and T. L. Ritchie, *Am. J. Trop. Med. Hyg.* 86, 46 (2012).
- S. Gunawan, *Bull. Health. Stud.* 13, 1 (1985).
- R. V. Sánchez, M. E. M. Núñez, and T. M. García, *IDCases* 2, 77 (2015).
- J. K. Baird, *Antimicrob. Agents Chemother.* 48, 4075 (2004).
- R. N. Price, E. Tjitra, C. A. Guerra, S. Yeung, N. J. White, and N. M. Anstey, *Am. J. Trop. Med. Hyg.* 77, 79 (2007).
- S. S. Yazdani, P. Mukherjee, V. S. Chauhan, and C. E. Chitnis, *Current Molecular Medicine* 6, 187 (2006).
- H. S. Seo, *Clin. Exp. Vaccine Res.* 4, 145 (2015).
- J. P. Hewitson, P. A. Hamblin, and A. P. Mountford, *Parasite Immunol.* 27, 271 (2005).
- S. K. Datta, S. Okamoto, T. Hayashi, S. S. Shin, I. Mihajlov, A. Fermin, D. G. Guiney, J. Fierer, and E. Raz, *Immunity* 25, 143 (2006).
- M. Alsharifi and A. Mulbacher, *Immunol. Cell Biol.* 88, 103 (2010).
- S. Datta, S. Roy, and M. Manna, *Braz. J. Infect. Dis.* 19, 36 (2015).
- F. Mwingira, G. Nkwangulila, S. Schoepflin, D. Sumari, H. P. Beck, G. Snounou, I. Felger, P. Olliaro, and K. Mugittu, *Malaria Journal* 10, 79 (2010).
- P. Gosi, C. A. Lanteri, S. D. Tynar, Y. Se, C. Lon, M. Spring, M. Char, D. Sea, S. Sriwichai, S. Surasri, S. Wongarunkochakorn, K. Pictana, D. S. Walsh, M. M. Fukuda, J. Manning, D. L. Saunders, and D. Bethell, *Malaria Journal* 12, 403 (2013).
- G. Snounou, X. Zhu, N. Siripoon, W. Jara, S. Thairithong, K. N. Brown, and S. Viriyakosol, *Trans. R. Soc. Trop. Med. Hyg.* 93, 369 (1999).
- K. Congpuong, R. Sukaram, Y. Prompan, and A. Dornae, *Asian Pacific Journal of Tropical Biomedicine* 4, 598 (2014).
- L. R. Pratt-Riccio, D. D. S. Perce-da-Silva, J. D. C. Lima-Junior, M. Theisen, F. Santos, C. T. Daniel-Ribeiro, J. D. O. Ferreira, and D. M. Banic, *Memórias Do Instituto Oswaldo Cruz* 108, 523 (2013).
- A. Auboy, F. Migot-Nabias, and P. Deloron, *Malaria Journal* 2, 12 (2003).
- G. Snounou and H. P. Beck, *Parasitol. Today* 14, 462 (1998).
- H. P. Beck, *Trop. Med. Int. Health* 4, 1 (1999).
- D. F. Clyde, H. Most, V. C. McCarthy, and J. P. Vanderberg, *Am. J. Med. Sci.* 266, 169 (1973).

25. D. Syafruddin, P. B. S. Asih, J. E. Siregar, and E. Tjitra, *Adv. Exp. Med. Biol.* 531, 103 (2003).
26. H. M. Gilles, H. M. Gilles, and D. A. Warrel, *Diagnostic Method in Malaria*, Oxford University Press, New York (1993), p. 79.
27. J. Wooden, S. Kyes, and C. H. Sibley, *Parasitol. Today* 9, 303 (1993).
28. H. P. Beck and I. Felger, *Methods and Techniques for Clinical Trials on Antimalarial Drug Efficacy: Genotyping to Identify Parasite Population* Amsterdam, The Netherlands, Medicines for Malaria Venture, Geneva (2007), p. 45.
29. M. Aregawi, R. Cibulskis, M. Otten, R. Williams, and C. Dye, *World Malaria Report 2008*, WHD Global Malaria Programme, World Health Organisation, Geneva (2008).
30. World Health Organization, *World Malaria Report Geneva, Switzerland (2011)*.
31. R. Kusriastuti, *Towards Malaria Elimination in Indonesia. Executive Board Meeting (2009)*.
32. L. S. Garver, A. C. Bahia, S. Das, J. A. Souza-Neto, J. Shiao, Y. Dong, and G. Dimopoulos, *PLoS. Pathog.* 8, 6 (2012).
33. L. Turner, C. W. Wang, T. Lavstsen, S. B. Mwakalinga, R. W. Sauerwein, C. C. Hemsden, and T. G. Theander, *PLoS ONE* 6, 12 (2011).
34. M. Karyana, L. Burdarm, S. Yeung, E. Kerangalem, N. Wariker, R. Maristela, K. G. Umana, R. Vemuri, M. J. Okoseray, P. M. Pentinan, P. Ebsworth, P. Sugianto, N. M. Anstey, E. Tjitra, and R. N. Price, *Malaria Journal* 7, 148 (2008).
35. P. B. S. Asih, I. E. Rozi, Herdiana, N. R. Pralama, A. P. N. Hidayati, and P. N. Anggi, *Malaria Journal* 11, 291 (2012).
36. A. Villa, J. Carmona-Fonseca, A. Benito, A. Martinez, and A. Maestre, *Infectio* 16, 37 (2012).
37. F. Huang, L. H. Tang, H. L. Yang, S. Zhou, H. Liu, J. Li, and S. Guo, *Malaria Journal* 11, 243 (2012).
38. R. Peek, T. Van Gool, D. Pnchoe, S. Greve, E. Bus, and L. Resida, *Am. J. Trop. Med. Hyg.* 73, 833 (2005).

Received: 21 August 2017. Accepted: 21 October 2017.