Genetic Diversity of *Plasmodium falciparum* Isolated from Yemen Based on the Genes of Merozoite Surface Proteins (*MSP-1* and *MSP-2*)

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A DISSERTATION
Submitted as partial fulfillment of the requirements for the degree of Master of Biotechnology

University Malaya

Faculty of science

2011

ABSTRACT

Malaria is a major health problem causing substantial morbidity and mortality with *Plasmodium falciparum* being the causative agent of the most severe and deadly form of malaria in humans. In Yemen Malaria is one of the most serious health problems. About 60% of the populations live in areas with malaria transmission. *P. falciparum* is the predominant species and accounts for more than 90% of malaria cases. Information on the nature and extent of genetic diversity within *P. falciparum* is essential for understanding the mechanism underlying the pathology of malaria, the acquisition of immunity, the spread of drug resistance and the condition of the transmission. This study was conducted in the high transmission area in Yemen (Taizz, Dhamar, and AL-Hudiydah) and was guided by the objective that were to establish molecular characterizations of field isolate *P. falciparum* in Yemen measured with polymorphic genetic markers of merozoite surface protein *msp-1* and *msp-2*.

Blood samples were screened for malaria parasites using Giemsa-stained thick and thin blood films. A total of 74 blood samples had *P. falciparum*, for which their *MSP-1* and *MSP-2* genes were studied using nested PCR. All the three families (K1, MAD20 and RO33) of *MSP-1* and the two families (FC27 and 3D7) of *MSP-2* were detected in this study. 3D7 allelic family was the most frequent (68%), followed by K1 (45%), RO33 (42%), FC27 (42%) and MAD20 (22%). The four allelic families, (MAD20, RO33, FC27 and 3D7), were significantly more prevalent in the hinterland areas as compared to coastland and highland areas ($p < 0.05$) of Yemen. The K1 allele type was most frequent in the highland ($p < 0.02$). The complexity of the infection was significantly ($p < 0.05$) the highest in the hinterland followed by coastland and highland. Urban areas had higher complexity of infection as compared to rural areas ($p < 0.05$). No significant difference was shown in the complexity of falciparum infection between the age groups ($p > 0.05$) nor the different levels of parasitaemia ($p > 0.05$). *MSP-2* had higher number of alleles than *MSP-1* (42 *vs* 18). The highest number of alleles of *MSP-1* and *MSP-2* was observed in the coastland and the rural areas.

In conclusion, significant differences in complexity and the distribution of the family alleles of *MSP-1* and *MSP-2* genes between hinterland, coastland and highland areas were observed, reflecting the intensity of malaria transmission between areas. This observation should be taken into consideration in implementing malaria control strategies in Yemen.

ABSTRAK

Malaria merupakan masalah kesihatan utama yang menyebabkan kadar kematian dan morbiditi yang tinggi. *Plasmodium falciparum* adalah spesies dominan yang merupakan agen penyebab malaria yang paling parah dan mengakibatkan kematian yang menyumbang kepada penderitaan manusia yang signifikan. Parasit intraseluler ini disebarkan di kalangan hos vertebrata oleh gigitan nyamuk *Anopheles* yang telah dijangkiti.Di Yemen, malaria merupakan satu masalah kesihatan yang amat serius. Sekitar 60% populasi penduduk hidup di kawasan transmisi malaria. *P. falciparum* adalah spesies yang dominan yang bertanggungjawab terhadap lebih dari 90% kes malaria.

Maklumat berkenaan kepelbagaian genetik dalam *P. falciparum* adalah penting bagi memahami mekanisma yang mendasari patologi malaria, kebolehan memperoleh immunisasi, penyebaran ubat ketahanan dan bagaimana transmisi berlaku. Kajian ini dijalankan di daerah yang mempunyai kadar transmisi malaria yang tinggi di Yemen (Taizz, Dhamar, dan AL-Hudiydah). Kajian ini adalah berdasarkan objektif bagi mengenalpasti sifat dan pencinan molekul isolat lapangan pencilan *P. falciparum* di Yemen yang diukur dengan penanda genetik polimorfik protein permukaan protein merozoit msp-1 dan *msp-2*.

Sampel darah diperiksa untuk mengenapasti kehadiran parasit malaria dengan menggunakan teknik pewarnaan Giemsa dengan filem darah tebal dan nipis. Dalam kajian ini sebanyak 74 sampel darah mempunyai *P. falciparum*, di mana gen *MSP-1* dan *MSP-2* dikaji menggunakan PCR tersarng. Kesemua tiga keluarga (K1, MAD20 dan RO33) dari *MSP-1* dan dua keluarga (FC27 dan 3D7) dari *MSP-2* berjaya dikesan dalam kajian ini. Keluarga alelik 3D7 adalah yang paling kerap dapat dikesan (68%), diikuti oleh K1 (45%), RO33 (42%), FC27 (42%) dan MAD20 (22%). Empat keluarga alelik (MAD20, RO33, FC27 dan 3D7), secara signifikan lebih banyak ditemui di daerah pendalaman berbanding dengan daerah pesisiran dan dataran tinggi ($p < 0.05$) di Yemen. Alel jenis K1 paling kerap ditemui di dataran tinggi ($p < 0.02$). Kerumitan jangkitan malaria adalah signifikan ($p < 0.05$) dan paling tinggi di daerah pendalaman diikuti oleh daerah penisiran dan dataran tinggi. Kawasan bandar mempunyai kerumitan jangkitan yang lebih tinggi jangkitan berbanding dengan kawasan luar bandar ($p < 0.05$). Tiada perbezaan yang signifikan ditunjukkan dalam kerumitan jangkitan falciparum antara kumpulan umur ($p > 0.05$) dan pelbagai peringkat parasitemia ($p > 0.05$). *MSP*-2 mempunyai jumlah alel yang lebih tinggi dari MSP1 (42 vs 18). Kadar tertinggi alel dari *MSP*-1 dan *MSP*-2 didapati di daerah penisiran dan kawasan luar bandar.

Kesimpulannya, perbezaan signifikan dalam kerumitan dan taburan keluarga alel gen *MSP*-1 dan *MSP*-2 antara kawasan pendalaman, daerah penisiran dan dataran tinggi yang dikaji, mengambarkan intensiti transmisi malaria di antara ketiga-tiga kawasan tersebut. Pemerhatian ini harus dipertimbangkan dalam pelaksanaan strategi kawalan malaria di Yemen.

ACKNOWLEDGEMENTS

First and foremost, I would to thank Allah for the blessing and the guidance, strength and courage to preserve throughout the duration of whole my life.

I would like to thank and extend my sincere gratitude and appreciation to my supervisor, Dr. Mohammed Mahday AL-Sharabi for his excellent support and guidance throughout the study. His enthusiasm, boundless ideas, expertise, experience, criticism, encouragement and challenges were very much appreciated.

I would also like to express my gratitude to my co-supervisor Prof. Dr. Fong Mun Yik for his assistance, guidance , and the opportunity to carry out my work in his laboratory . I would like to express great thanks to my co-supervisor Dr. Nazia Abdul-Majed for her assistance ,advice, guidance and comments throughout the study.

Special thanks to the head and staff of Department of Parasitology, Faculty of Medicine, for their support. I thank University of Malaya for supporting the research under the postgraduate grant (PPP) no.P0083/2010B. I would like to thank all my lecturers in the faculty of science, colleagues, and friends for their unending encouragements. I am grateful for Mr Abdulsalam Mohammed Qasem Al-mekhlafi for sharing with me the field samples and his kind help. I would like to thank all members of molecular laboratory of Parasitology Department and my close friend Mustafaa Kassim,Ahmed Gumel, Fahad Farid and Farid Amir for their encouragement

Most importantly, I would like to thank my parents, my brothers, my sisters, and my wife for supporting me. It is through their encouragement, love, support and prayers that I have

made it through all the steps to reach this point in life, and I could not have done it without them.

Special thank to my sister Amal she didn't skimp on her time and she sacrifice her time in order to provide advice and assistance me in different situation during my studies.

I am also thankful to the Higher Education Ministry of my country for the support,given to me the scholarship to study in Malaysia.

Finally, I would like to thank everyone for putting up with me for the last several years. I hope that this dissertation has made some contribution to the field of malaria diversity and I hope that everyone who reads this dissertation finds it useful in their work.

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
bp	Base pair
CDC	Centre for Disease Control
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra acetic acid
EIR	Entomological inoculation rate
IPT	Intermittent preventive treatment
MSP-1	Merozoite surface protein 1
PCR	Polymerase chain reaction
RBC	Red blood cell
U	Unit
UV	Ultraviolet
WHO	World Health Organization
RDT	Rapid diagnostic test
HRP-2	Histidine-rich protein 2
GLURP	Glutamate-rich protein
RT	Reverse transcriptase
MS	Microsatellite analysis
MALDI-TOF	Matrix- assisted laser desorption-ionization time of flight mass spectrometry
MVR	Minisatellite variant repeat
LDR-FMA	Ligase detection reaction-fluorescent microsphere assay
GPI	glycosylphosphatidylinositol
TEA	Tris-EDTA
SPSS	Statistical Package for the Social Sciences
MOI	Multiplicity of infection
USA	United States of America
NMCP	National Malaria Control Programme
EGF	Epidermal growth factor

DEFINITIONS

Definitions of terms used through this thesis:-

Allele: one of the several alternative forms of genes that occupy the same locus.

Allelic type: alleles of a gene that can be grouped based on similar characteristics e.g. sequence similarity of the allelic types of *MSP-1* and *MSP-2*, also referred to as allelic families.

Genotype: combination of alleles that determine a particular genetic characteristic.

Infection diversity: the number of clones detected within one sample. This number represents the minimum number of circulating clones; also referred as genetic diversity of infections or multiplicity of infection.

Parasitaemia: is the quantitative content of parasites in the blood. It is used as a measurement of parasite load in the organism and an indication of the degree of an active parasitic infection.

Rural areas: are large and isolated areas of an open country with low population density.

An urban area: is characterized by higher population density and vast human features in comparison to areas surrounding it.

DEDICATION

To all of you who made this possible, thank you