UNIVERSITI PUTRA MALAYSIA

MOLECULAR CHARACTERIZATION AND ISOENZYME PROFILES OF GIARDIA DUODENALIS ISOLATES FROM IRANIAN PATIENTS IN FARS PROVINCE, IRAN

MOHAMMAD RAYANI

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By

MOHAMMAD RAYANI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

January 2014

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Dedicated to:

The memory of my late Father, and Mother,

My loving Wife and children,

&

All my supportive family members

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

MOLECULAR CHARACTERIZATION AND ISOENZYME PROFILES OF *GIARDIA DUODENALIS* ISOLATES FROM IRANIAN PATIENTS IN FARS PROVINCE, IRAN

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January 2014

Chairman: Ngah Zasmy Unyah, Ph.D

Faculty: Medicine and Health Sciences

Giardia duodenalis is the most common intestinal parasite among humans and is endemic throughout the world. Diarrhea and malnutrition are the main clinical pathogenesis especially in children. Giardiasis is one of the common infections found occurring in Iran. Identifying the prevalence of the common genotype assemblages and zymodemes of *G. duodenalis* in this province will allow for a better understanding of the route and source of the transmission for *G. duodenalis*, especially related to the control and prevention strategies. The heterogeneity among *G. duodenalis* strains may explain the variable clinical manifestations, host response and treatment efficacy characteristic of human giardiasis. The first objective was to study the genetic characterization of *G. duodenalis* isolates at Fars Province, south of Iran by semi-nested PCR and isoenzyme analyses. The second objective was to identify the most common *G. duodenalis* assemblages or sub-assemblages involved in the transmission of giardiasis in this area. Human fecal samples (n=1000) were collected from health centers and hospitals in Fars province, south of Iran from September 2009 to August 2010. Standard fecal staining method and microscopic confirmation of both *G. duodenalis* cysts and trophozoites were performed before and after the fecal concentration method. Purification and isolation of *G. duodenalis* cysts and trophozoites were based on the modification of the standard sucrose gradient method. Consequently, DNA was extracted using the standard Phenol Chloroform Isoamyl Alcohol method. A fragment of the SSU-rDNA (292 bp) gene was amplified using the forward primer RH11 and reverse primer RH4. Semi-nested PCR and sequence analysis were then performed using primers GDHeF, GDHiF, and GDHiR that amplifies a 432 bp fragment of the glutamate dehydrogenase gene (*gdh*). Phylogenetic analysis was carried out using a neighbor-joining tree composed of the 40 nucleotide sequences of successfully isolated *G. duodenalis* and compared with the known sequences published in GenBank. Fifteen cultures of *G. duodenalis* isolates were analyzed using isoenzyme in a polyacrylamide gel electrophoresis (PAGE). Five different enzyme systems were

used to characterize each isolate: (i) Glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.444), (ii) Glucose phosphate isomerase (GPI, E.C. 5.3.1.9), (iii) Malate dehydrogenase (MDH, E.C. 1.1.1.37), (iv) Malic enzyme (ME, E.C. 1.1.1.40) and (v) Phosphoglucomutase (PGM, E.C. 2.7.5.1). The results indicated that 107/1000 (10.7%) samples were found positive for *G. duodenalis* based on microscopy confirmation. Almost similar results were observed in molecular study and isoenzyme profile analysis. PCR analysis identified 80% (40/50) samples were positive for *G. duodenalis* based on SSU-rDNA amplification on sucrose gradient samples. Further genotyping has resulted in 80% (32/40 samples) isolates as sub-assemblage of AII and 20% (8/40 samples) isolates as assemblage B based on the DNA sequence of the *gdh*. Phylogenetic analysis had shown that *G. duodenalis* isolates at Fars province were widely distributed within assemblage A cluster (sub-assemblage AII) and the remaining isolates were dispersed throughout the assemblage B cluster (sub-assemblage BIII and BIV). Electrophoretic heterogeneity was found in *G. duodenalis* enzymes profile. One identical isozyme was detected for G6PD isoenzyme pattern. Two different isozymes were detected for GPI and MDH isoenzyme patterns. In addition, three different isozymes were detected for ME and PGM isoenzyme patterns. Further analysis has shown that four zymodemes were found among the fifteen isolates of *G. duodenalis*. The zymodemes 1, 2, 3 and 4 was observed to have similarity with 7, 2, 4 and 2 isolates, respectively. In conclusion, five isoenzyme systems were used in this study; these are G6PD, GPI, MDH, ME and PGM for the characterization of *G. duodenalis* isolates and distinguish zymodemes of the parasite in Iran. The isoenzyme electrophoretic profiles divided fifteen *G. duodenalis* isolates into four zymodemes and revealed genetic heterogeneity between the Iranian isolates. These variations are related to the clinical manifestation, pathogenicity, drug susceptibility and host specificity. G6PD isoenzyme pattern had the most homogeneity, while ME and PGM isoenzyme pattern had the most heterogeneity in our study. The present study showed that *G. duodenalis* sub-assemblage AII was the predominant assemblage in Fars Province. This indicates an anthroponotic transmission from human to human was one of the main causes of giardiasis in this area. Health promotion, public education, improving sanitation conditions, personal hygiene, improving clean drinking water and food are important strategies that should be addressed to control and prevent giardiasis. Through this study, potentially important and useful data on the distribution of different genotypes and isoenzymes profiles of *G. duodenalis* in Iran were obtained. These data represent a significant advancement in the current understanding of the transmission of *G. duodenalis* assemblages in Iran and could aid in future studies for epidemiology, clinical management and prevention purposes.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN MOLEKUL DAN PROFIL ISOENZIM ISOLAT *GIARDIA DUODENALIS* DARIPADA PESAKIT DI WILAYAH FARS, IRAN

Oleh

MOHAMMAD RAYANI

Januari 2014

Pengerusi: Ngah Zasmy Unyah, Ph.D

Fakulti: Perubatan dan Sains Kesihatan

Giardia duodenalis adalah merupakan parasit yang menjangkiti usus kebanyakan manusia dan endemik di serata dunia. Cirit-birit dan kekurangan zat makanan merupakan simptom klinikal yang utama khususnya berlaku pada kanak-kanak. Objektif pertama adalah mempelajari pencirian genetik terhadap pencilan *G. duodenalis* di Wilayah Fars di Selatan Iran dengan mengunakan kaedah PCR dan isoenzim. Objektif kedua adalah mengenalpasti jenis asemblaj atau sub-asemblaj *G. duodenalis* yang lazimnya didapati terlibat dengan transmisi penyakit giardiasis di wilayah tersebut. Sampel tinja manusia (n=1000) telah diperolehi dari pusat kesihatan dan hospital di Wilayah Fars di Selatan Iran bermula dari September 2009 hingga Ogos 2010. Kaedah pewarnaan tinja dan pengecaman mengunakan mikroskop bagi mengenalpasti sista dan tropozoit telah dilakukan sebelum dan selepas kaedah konsentrasi tinja dijalankan. Purifikasi dan isolasi terhadap sista dan tropozoit *G. duodenalis* adalah berdasarkan modifikasi kaedah kecerunan sukros. DNA ekstrak dilakukan mengunakan kaedah fenol klorofom isoamilalkohol (PCI) dan peningkatan PCR dijalankan terhadap SSU-rDNA (292bp) mengunakan primer kehadapan iaitu RH11 dan primer kebelakang iaitu RH4. Kaedah PCR tersarang dilakukan dengan mengunakan primer GDHeF, GDHiF, dan GDHiR bagi peningkatan serpihan gen *gdh* kepada 432bp. Analisi filogenetik dijalankan mengunakan 'neighbor-joining tree' merangkumi 40 jujukan nukleotid dari *G. duodenalis* yang telah berjaya di asingkan berdasarkan jujukan yang telah sedia ada di GenBank. Lima belas pencilan *G. duodenalis* telah dianalisis mengunakan isoenzim elektroforesis gel poliakrilamid (PAGE). Lima jenis sistem enzim yang berbeza juga digunakan bagi perincian setiap pencilan, seperti: (i) Glukos-6-fosfat dehidrogenas (G6PD, E.C. 1.1.1.444), (ii) Glukos fosfat isomeras (GPI, E.C. 5.3.1.9), (iii) Malat dehidrogenas (MDH, E.C. 1.1.1.37), (iv) Malic enzim (ME, E.C. 1.1.1.40) dan (v) Fosfoglukomutas (PGM, E.C. 2.7.5.1). Hasil kajian mendapati sebanyak 107/1000 (10.7%) dari sampel adalah positif terhadap *G. duodenalis* berdasarkan pengecaman mengunakan kaedah mikroskopi. Kaedah peningkatan PCR telah dapat mengenal pasti sebanyak 80% (40/50) sampel

positif terhadap *G. duodenalis* berdasarkan peningkatan SSU-rDNA. Keputusan kaedah penjenisanmenunjukkansebanyak 32/40 (80%) pencilan adalah dari asemblaj AII manakala sebanyak 8/40 (20%) pencilan dikenal pasti sebagai asemblaj B berdasarkan jujukan DNA dari *gdh*. Analisis kaedah penjenisan menunjukkan taburan pencilan terhadap *G. duodenalis*adalah tertumpu kepada asemblaj A (khususnya sub-asemblaj AII), manakala pencilan selebihnya didapati lebih tertumpu kepada asemblaj B (khususnya sub-asemblaj BIII dan BIV). Satu isoenzim yang menyerupai corak isoenzim G6PD juga telah dikenal pasti. Manakala, dua jenis isoenzim yang menghasilkan corak isoenzim menyerupai isoenzim GPI dan MDH turut dikenalpasti. Terdapat juga tiga jenis isoenzim yang menyerupai corak isoenzim ME dan PGM juga telah dikenal pasti. Sejumlah empat jenis zimodem telah dikenal pasti daripada 15 pencilan *G. duodenalis* iaitu zimodem nombor 1 mempunyai 7 pencilan, zimodem nombor 2 mempunyai 2 pencilan, zimodem nombor 3 mempunyai 4 pencila dan zimodem nombor 4 mempunyai 2 pencilan. Kesimpulannya, hasil kajian ini mendapati *G. duodenalis* sub-asemblaj AII merupakan jenis asemblaj yang paling dominan di Wilayah Fars dan profil elektroforesis terhadap isoenzim menunjukkan kepelbagaian genetik wujud pada pencilan *G. duodenalis* dari Iran. Ini menunjukan yang transmisi secara artroponotik iaitu dari manusia ke manusia adalah merupakan penyebab utama giardiasis di wilayah ini. Data yang diperolehi dari kajian ini akan dapat meningkatkan pemahaman yang sedia ada mengenai transmisi asemblaj *G. duodenalis* di Iran.

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I certify that ……

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 March 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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