



UNIVERSITI PUTRA MALAYSIA

**PREVALENCE, CLINICO-EPIDEMOIOLOGICAL CHARACTERISTICS
AND RISK FACTORS OF INTESTINAL MICROSPORIDIOSIS IN
CHILDREN WITH MALIGNANCY IN THE INSTITUTE OF
PEDIATRIC, KUALA LUMPUR, MALAYSIA**

NUR RAIHANA BINTI ITHNIN

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MALAYSIA**



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
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**PREVALENCE, CLINICO-EPIDEMIOLOGICAL CHARACTERISTICS AND
RISK FACTORS FOR INTESTINAL MICROSPORIDIOSIS IN CHILDREN
WITH MALIGNANCY IN THE INSTITUTE OF PAEDIATRIC, KUALA LUMPUR
MALAYSIA**

By

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March 2013

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Intestinal microsporidiosis is an infection caused by Microsporidia, an obligate intracellular protozoan parasite that have a unique belt-like striped structure of the spore. The common species that infect human are *Enterocytozoon bieneusi* and *Encephalitozoon* spp. This infection common in immunocompromised individuals especially in HIV patients. Nonetheless, data from other immunocompromised individuals such as children, elderly, patients undergoing immunosuppressive therapy are still scanty. On the other hand, it had been widely reported in immunocompetent population currently. The prevalence of microsporidiosis varies and it depends on the use of diagnostic methods to detect the spore in the sample, the geographic distribution and the type of studied population. The aim of the study was to determine the prevalence of microsporidiosis in malignancy children population in Institute of Pediatric,

Malaysia and to determine the possible risk factors that might be associated with microsporidiosis. In addition, the performance of Calcofluor White M2R (CFW) fluorescence staining compared with Modified Gram-chromotrope Kinyoun (MGCK) staining also will be determined. This cross sectional study was conducted from November 2009 until August 2010 in KK3 Oncology ward, Institute of Pediatrics, Hospital Kuala Lumpur. One hundred and six stool (n=106) samples from children with different types of malignancies were collected from the ward. The standard proforma was distributed to the children or caregivers or parents to determine the possible risk factors that might be associated with microsporidiosis. The stool samples were stained with CFW and MGCK staining. The prevalence rate of microsporidiosis in children with malignancies was 77.4% which 82 out of 106 samples were positive of microsporidia spores. Data from the sociodemographic characteristics did not present any significant association with microsporidiosis. Children with hematological cancer were the dominant group with microsporidiosis (64.2%). The clinical manifestations that associated with microsporidiosis were vomiting (χ^2 value: 8.048, p value: 0.005, OR: 0.290, 95% CI: 0.099-0.851) and stool without mucous (χ^2 value: 4.138, p value: 0.042, OR: 3.200, 95% CI: 1.002-10.224). Multiple logistic regression analysis showed that children presented with vomiting were 6.564 times more likely to have microsporidiosis compared to those who had not ($\beta=1.182$, SE=0.732, Wald $\chi^2=6.614$, p value=0.010, OR=6.564, 95% CI=1.565-27.539). The clinical symptoms which occurred in malignancy children were uncertain whether it is caused by microsporidiosis

itself or treatment that they encountered with. The environmental risk factors did not contribute to any significant association with microsporidiosis (p value > 0.05). The evaluation of CFW staining performance with the reference standard method which was MGCK staining for detection of microsporidia spore in sample was done. The sensitivity of CFW staining was high (95.5%) but the specificity was very low (4.3%). Hence, it would contribute to a flaw in the identification of microsporidia. As a conclusion, the study gives a baseline data for microsporidia infection in malignancy children in Malaysia. Therefore, the further study is needed in term to develop the better prevention and control against microsporidiosis in malignancy children. Moreover, the corroboration between CFW and MGCK staining methods can be apply in clinical service as a routine screening method for the detection of microsporidia infection in patient samples.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PREVALENS, CIRI-CIRI KLINIKO-EPIDEMIOLOGI DAN FAKTOR-FAKTOR YANG BERISIKO JANGKITAN MIKROSPORIDIA USUS DI KALANGAN KANAK-KANAK YANG MENGHIDAPI MALIGNANSI DI INSTITUT PEDIATRIK, KUALA LUMPUR, MALAYSIA

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Mikrosporidiosis usus adalah sejenis jangkitan yang disebabkan oleh Mikrosporidia, parasit protozoa yang obligat intrasel. Spesies yang lazim menjangkiti manusia adalah *Enterocytozoon bieneusi* dan *Encephalitozoon* spp. Jangkitan oleh parasit ini lazimnya terlibat pada individu yang terimunokompromi terutama pada pesakit yang menghidapi HIV. Namun, data daripada golongan individu yang terimunokompromi selain daripada pesakit AIDS seperti kanak-kanak, orang lanjut usia dan pesakit yang menjalani terapi immuno yang terhalang masih lagi kurang. Malahan, jangkitan ini juga telah dilaporkan secara meluas di dalam populasi yang terimunokompeten pada masa kini. Prevalens mikrosporidiosis adalah berbeza-beza kerana ianya bergantung kepada kaedah diagnosis yang digunakan untuk mengecam sporanya di dalam sampel, kawasan geografi yang meluas dan latar belakang populasi yang dikaji. Tujuan kajian ini dijalankan adalah untuk

menentukan prevalens mikrosporidiosis pada populasi kanak-kanak yang menghidapi malignansi di Malaysia dan mengenalpasti faktor-faktor risiko yang berkemungkinan dalam menentukan perkaitan antara faktor-faktor tersebut dengan mikrosporidiosis. Selain daripada itu, pencapaian teknik pewarnaan Calcofluor White M2R fluoresen (CFW) juga akan dibandingkan dengan teknik pewarnaan Gram-chromotrope Kinyoun terubahsuai (MGCK). Kajian keratan rentas ini telah dijalankan dari November 2009 sehingga Ogos 2010 di wad Onkologi, Institut Pediatrik, Hospital Kuala Lumpur. Sebanyak satu ratus enam sampel tinja telah diperolehi daripada kanak-kanak yang menghidapi pelbagai jenis malignansi dan juga telah memenuhi kriteria yang dikehendaki dalam kajian ini. Soal selidik yang bertaraf standard telah diedarkan kepada kanak-kanak tersebut untuk menentukan faktor-faktor risiko yang berkemungkinan akan mempunyai kaitan dengan mikrosporidiosis. Seterusnya, kaedah pewarnaan CFW dan MGCK akan digunakan ke atas sampel-sampel yang diperolehi untuk mengenalpasti kehadiran spora mikrosporidia. Kadar prevalens bagi mikrosporidiosis adalah 77.4% iaitu 82 daripada 106 sampel adalah positif terhadap spora mikrosporidia. Data dari ciri-ciri sosiodemografi tidak menunjukkan sebarang perkaitan yang signifikan terhadap mikrosporidiosis. Kanak-kanak yang menghidapi malignansi darah merupakan kumpulan yang dominan untuk dijangkiti oleh mikrosporidiosis (64.2%). Ciri-ciri klinikal yang berkait dengan jangkitan mikrosporidiosis adalah muntah (nilai χ^2 : 8.048, nilai p : 0.005, OR: 0.290, 95% CI: 0.099-0.851) dan tinja tanpa mukus (nilai χ^2 : 4.138, nilai p : 0.042, OR: 3.200, 95% CI: 1.002-10.224). Ujian logistik regresi menunjukkan kanak-kanak yang mengalami muntah adalah 6.564 kali lebih cenderung untuk dijangkiti oleh

mikrosporidiosis berbanding dengan kanak-kanak yang tidak mengalaminya ($\beta=1.182$, SE=0.732, Wald $\chi^2=6.614$, nilai $p =0.010$, OR=6.564, 95% CI=1.565-27.539). Simptom-simptom klinikal yang dialami oleh kanak-kanak yang menghidapi malignansi ini masih tiada kepastian sama ada ciri-ciri klinikal tersebut adalah punca sebenar yang menyebabkan jangkitan mikrosporidia atau pun ianya kesan daripada rawatan yang diterima. Faktor-faktor persekitaran yang berisiko pula tidak menyumbang kepada perkaitan yang signifikan kepada mikrosporidiosis (nilai $p>0.05$). Penilaian terhadap pencapaian kaedah pewarnaan CFW dengan berpandukan kaedah pewarnaan MGCK sebagai kaedah rujukan yang standard telah diperolehi dalam mengenlpasti spora mikrosporidia di dalam sampel. Sensitiviti kaedah pewarnaan CFW fluoresen adalah tinggi (95.5%) tetapi spesifisitinya terlalu rendah (4.3%). Oleh itu, ia boleh menyebabkan akan berlakunya kesilapan di dalam pengenalpastian mikrosporidia. Kesimpulannya, kajian ini memberi asas mengenai jangkitan mikrosporidia di kalangan kanak-kanak yang menghidapi malignansi di Malaysia. Oleh itu, kajian pada masa hadapan adalah diperlukan dalam membangunkan kaedah pengawalan dan pencegahan yang lebih berkesan ke atas mikrosporidiosis di kalangan kanak-kanak yang menghidapi malignansi. Selain dari itu, koroborasi di antara kaedah pewarnaan CFW dan MGCK boleh dijadikan sebagai satu kaedah imbasan yang dilakukan secara rutin di bahagian klinikal servis untuk mengenalpasti kehadiran jangkitan mikrosporidia pada sampel pesakit.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or is not concurrently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NUR RAIHANA BINTI ITHNIN

Date: 28 MARCH 2013



LIST OF TABLES

| Table | | Page |
|-------|---|------|
| 1 | Morphological characteristics of microsporidia spp. in human | 28 |
| 2 | Animal hosts of human microsporidia | 38 |
| 3 | Possible sites of infection for micorsporidia | 45 |
| 4 | Various diagnostic methods for microsporidia detection | 60 |
| 5 | General characteristics of patients participated in the study | 77 |
| 6 | Prevalence of microsporidiosis amongst children with malignancies | 78 |
| 7 | Prevalence of microsporidiosis in children with malignancies in relation to gender, age group, races and region | 78 |
| 8 | Association of microsporidiosis in relation to gender | 78 |
| 9 | Association of microsporidiosis in relation to race | 79 |
| 10 | Association of microsporidiosis in relation to age | 79 |
| 11 | The classification of cancer according to ICD-O-3 for oncology | 90 |
| 12 | The association between microsporidiosis and the types of malignancy | 94 |
| 13 | The association of microsporidiosis with different types of hematological cancers and disorders | 94 |
| 14 | Association of microsporidiosis with clinical manifestations | 97 |
| 15 | Relationship between microsporidiosis and clinical presentations | 98 |
| 16 | Association of microsporidiosis with hematological profiles | 98 |
| 17 | Distribution of potentially pathogenic microbial organisms isolated from blood of children | 99 |
| 18.1 | Association of microsporidiosis with different types of meat | 101 |

| | | |
|------|--|-----|
| 18.2 | Association of microsporidiosis with different egg-based food | 101 |
| 18.3 | Association of microsporidiosis with different types of drinks consumed by children | 102 |
| 18.4 | Association of microsporidiosis with children who had contact with pets | 102 |
| 18.5 | Association of microsporidiosis with water outdoor activities | 103 |
| 18.6 | Association of microsporidiosis with history of traveling by children | 104 |
| 18.7 | Association of microsporidiosis with hygienic practices amongst children | 104 |
| 18.8 | Association of microsporidiosis with children who had history of a nursery care | 104 |
| 18.9 | Association of microsporidiosis with children referred from other hospitals | 104 |
| 19 | Calculation for the characteristics of diagnostic test | 123 |
| 20 | The formula for the characteristics of diagnostic test | 123 |
| 21 | The ability of Calcofluor White M2R and Modified Gram-chromotrope Kinyoun stainings in detection of microsporidial spores in stool samples | 124 |
| 22 | Performance of Calcofluor White M2R fluorescence with Modified Gram-chromotrope Kinyoun staining as the reference standard | 128 |
| 23 | Characteristics of Calcofluor White M2R diagnostic performance | 128 |
| 24 | Grading of microsporidial spores | 128 |

LIST OF FIGURES

| Figure | | Page |
|---------------|--|-------------|
| 1 | Taxonomy of human-infecting human microsporidia | 18 |
| 2 | Morphology of microsporidial spore | 22 |
| 3 | Life cycle of microsporidia in humans | 22 |
| 4 | Geographic distribution of microsporidia infection | 34 |
| 5 | Transmission electron micrograph of <i>Encephalitozoon hellem</i> | 62 |
| 6 | <i>Encephalitozoon cuniculi</i> spores in conjunctival swab from an HIV-infected patient in disseminated infection | 63 |
| 7 | Calcofluor white staining of microsporidial spores in urine sample | 63 |
| 8 | Microsporidial spores in Calcofluor White M2R stain | 125 |
| 9 | Microsporidial spores measuring 1.0µm x 2.0µm approximately in modified Gram-chromotrope Kinyoun stain with grading 1+ | 126 |
| 10 | Microsporidial spores measuring 1.0µm x 2.0µm approximately in modified Gram-chromotrope Kinyoun stain with grading 2+ | 127 |
| 11 | Front door of KK3 oncology ward | 193 |
| 12 | Treatment room in KK3 ward | 193 |
| 13 | The whole environment in KK3 ward | 194 |

TABLE OF CONTENTS

| | Page | |
|--------------------------|---|----|
| ABSTRACT | iii | |
| ABSTRAK | vi | |
| ACKNOWLEDGEMENTS | ix | |
| APPROVAL | x | |
| DECLARATION | xii | |
| LIST OF TABLES | xiii | |
| LIST OF FIGURES | xiv | |
| GLOSSARY OF TERMS | xvi | |
| | | |
| CHAPTER | | |
| 1 | INTRODUCTION | 1 |
| | | |
| 2 | LITERATURE REVIEW | 15 |
| 2.1 | Definition and history | 15 |
| 2.2 | Taxonomy | 16 |
| 2.3 | Morphology of microsporidia | 19 |
| 2.4 | Life cycle | 20 |
| 2.5 | Pathogenesis | 23 |
| 2.6 | Genus and species specific characteristic | 24 |
| 2.6.1 | <i>Enterocytozoon bieneusi</i> | 24 |
| 2.6.2 | <i>Encephalitozoon</i> spp. | 25 |
| 2.6.3 | <i>Septata intestinalis</i> | 26 |
| 2.7 | Epidemiology | 29 |
| 2.7.1 | Geographic distribution | 29 |
| 2.7.2 | Prevalence of microsporidiosis in HIV/AIDS infected patients | 29 |
| 2.7.3 | Prevalence of microsporidiosis in other immunocompromised patients | 31 |
| 2.7.4 | Prevalence of microsporidiosis in other immunocompetent individuals | 32 |
| 2.7.5 | Prevalence in Malaysia | 34 |
| 2.7.6 | Sources and modes of transmission | 35 |
| 2.8 | Clinical manifestations | 42 |
| 2.9 | Diagnostic methods | 48 |
| 2.9.1 | Light microscopy examination | 49 |
| 2.9.2 | Cytologic diagnosis | 53 |
| 2.9.3 | Histologic examination | 54 |
| 2.9.4 | Electron microscopic detection | 55 |
| 2.9.5 | Immunofluorescence detection | 56 |
| 2.9.6 | Molecular technique | 57 |
| 2.9.7 | Cell culture | 59 |
| 2.9.8 | Serology | 59 |
| 2.10 | Therapy and prevention | 64 |

| | | |
|----------|---|-----|
| 3 | PREVALENCE OF INTESTINAL MICROSPORIDIOSIS AND ITS ASSOCIATION WITH SOCIO DEMOGRAPHIC CHARACTERISTICS OF CHILDREN WITH MALIGNANCIES IN MALAYSIA | 66 |
| 3.1 | Introduction | 66 |
| 3.2 | Methodology | 69 |
| 3.2.1 | Study area | 69 |
| 3.2.2 | Study design | 69 |
| 3.2.3 | Study population | 69 |
| 3.2.4 | Sampling frame | 69 |
| 3.2.5 | Sampling method | 70 |
| 3.2.6 | Sample size | 71 |
| 3.2.7 | Study instrument | 73 |
| 3.2.8 | Statistical analysis | 73 |
| 3.2.9 | Ethical consideration | 73 |
| 3.3 | Results | 74 |
| 3.3.1 | Background of the study place | 74 |
| 3.3.2 | Background characteristics of patients | 75 |
| 3.3.3 | Prevalence of intestinal microsporidiosis and its association with socio demographic factors amongst children with malignancies | 77 |
| 3.4 | Discussion | 80 |
| 3.5 | Conclusion | 84 |
| 4 | CLINICAL CHARACTERISTIC AND RISK FACTORS OF INTESTINAL MICROSPORIDIOSIS AMONGST HOSPITALIZED CHILDREN WITH MALIGNANCIES | 85 |
| 4.1 | Introduction | 85 |
| 4.2 | Methodology | 88 |
| 4.2.1 | Selection of children | 89 |
| 4.2.2 | Questionnaire | 90 |
| 4.2.3 | Sample preparation | 91 |
| 4.2.4 | Statistical analysis | 93 |
| 4.3 | Results | 93 |
| 4.3.1 | Association of types of malignancies with microsporidiosis | 93 |
| 4.3.2 | Association of clinical presentations with microsporidiosis | 95 |
| 4.3.3 | Association of risk factors with microsporidiosis | 99 |
| 4.4 | Discussion | 105 |
| 4.5 | Conclusion | 114 |

| | | |
|-----------------------------|---|-----|
| 5 | EVALUATION OF CALCOFLUOR WHITE M2R FLUORESCENCE AND MODIFIED GRAM CHROMOTROPE KINYOUN STAINING TECHNIQUES FOR DETECTING MICROSPORIDIAL SPORES IN FECAL SAMPLES | 115 |
| 5.1 | Introduction | 115 |
| 5.2 | Methodology | 118 |
| 5.2.1 | Study population | 118 |
| 5.2.2 | Fecal sample collection | 119 |
| 5.2.3 | Fecal smear preparation | 119 |
| 5.2.4 | Calcofluor White M2R fluorescence staining technique | 120 |
| 5.2.5 | Modified Gram-chromotrope Kinyoun staining technique | 121 |
| 5.2.6 | Statistical analysis | 122 |
| 5.3 | Results | 123 |
| 5.3.1 | Descriptive analysis of Calcofluor White M2R and modified Gram-chromotrope Kinyoun staining methods | 123 |
| 5.3.2 | Characteristics of the microsporidial spores | 124 |
| 5.3.3 | Diagnostic performance of Calcofluor White M2R in the detection of microsporidial spores | 127 |
| 5.3.4 | Microsporidial grading | 128 |
| 5.4 | Discussion | 129 |
| 5.5 | Conclusion | 133 |
| 6 | SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH | 134 |
| REFERENCES | | 137 |
| APPENDICES | | 170 |
| BIODATA OF STUDENT | | 195 |
| LIST OF PUBLICATIONS | | 196 |