UNIVERSITI SAINS MALAYSIA
PROJEK PENYELIDIKAN JANGKA PENDEK
LAPORAN AKHIR

A STUDY OF LYMPHOCYTE SUBSETS, ACTIVATION MARKERS AND HERPERS VIRUSES INFECTION IN EPILEPTIC PATIENTS IN HUSM

PENYELIDIK

DR. WAN ZURAIDA WAN AB. HAMID

PENYELIDIK BERSAMA

PROF. MUSTAFFA MUSA
DR. FAUZIAH MOHD IDRUS
DR. SHALINI BHASKAR

2011

RU07(27)

UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT
FINAL REPORT
Geran Penyelidikan Universiti
Laporan Akhir

A.	**TITLE OF RESEARCH:** *Tajuk penyelidikan:* **A STUDY OF LYMPHOCYTE SUBSETS, ACTIVATION MARKERS AND HERPES VIRUSES INFECTION IN EPILEPTIC PATIENTS IN HUSM.**
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader:** *Nama Ketua Penyelidik:* DR. WAN ZURAIDA WAN AB. HAMID
	Name of Co-Researcher *Nama Penyelidik Bersama:* PROF. MUSTAFFA MUSA DR. FAUZIAH MOHD IDRIS DR. SHALINI BHASKAR
(ii)	**School/Institute/Centre/Unit :** *Pusat Pengajian /Institut/Pusat/Unit :* SCHOOL OF MEDICAL SCIENCES / IMMUNOLOGY DEPARTMENT

C. **Research Platform (Please tick (/) the appropriate box):**
Pelantar Penyelidikan (Sila tanda (/) kotak berkenaan):

- ☐ **A. Life Sciences**
  Sains Hayat
- ☐ **B. Fundamental**
  Fundamental
- ☐ **C. Engineering & Technology**
  Kejuruteraan & Teknologi
- ☐ **D. Social Transformation**
  Transformasi Sosial
- ☐ **E. Information & Communications Technology (ICT)**
  Teknologi Maklumat & Komunikasi
- ☑ **F. Clinical Sciences**
  Sains Klinikal
- ☐ **G. Biomedical & Health Sciences**
  Bioperubatan Sains Kesihatan

D. **Duration of this research :**
Tempoh masa penyelidikan ini :

***Duration :** 35 months
Tempoh :

From : October 2007 **To** : August 2010
Dari: *Ke :*

E. **ABSTRACT OF RESEARCH**

(An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English.**
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Please refer to attachment

Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**
Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

F. **SUMMARY OF RESEARCH FINDINGS**
Ringkasan dapatan Projek Penyelidikan

- Epileptic patients had their lymphocyte's subset marker, CD16+56 (NK cells) to be significantly higher (*p* value: 0.042) and activated marker, $CD8^+CD25^+$, were significantly lower (*p* value: 0.008) compared to healthy controls.
- $CD16^+56^+$, $CD4^+CD25^+$ and the patient's education level, were the only factors that were significantly associated to epilepsy.
- No significant correlation (*p* value > 0.05) was observed between all lymphocyte subsets and herpes viruses infections, however,
- CMV IgG showed a statistically significant difference in epileptic patients as compared to healthy controls with $p < 0.001$

G. **COMPREHENSIVE TECHNICAL REPORT**
Laporan Teknikal Lengkap
Applicants are required to prepare a comprehensive technical report explaining the project.
(This report must be attached separately)
Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Laporan ini mesti dikepilkan]

List the key words that reflectour research:
Senaraikan kata kunci yang mencerminkan penyelidikan anda:

English	Bahasa Malaysia
Epilepsy	Epilepsi
Herpes viruses	Virus-virus herpes
Lymphocyte subsets	Subset limfosit
Activation marker	Penanda pengaktifan
Immunoglobulin	Imunoglobulin

H.

a) Results/Benefits of this research
Hasil Penyelidikan

No. *Bil:*	Category/Number: *Kategori/ Bilangan:*	Promised	Achieved
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)*	1	1
2.	**Human Capital Development**		
	a. Ph. D Students		
	b. Masters Students	**1**	**1**
	c. Undergraduates (Final Year Project)		
	d. Research Officers		
	e. Research Assisstants		
	f. Other: Please specify		
3.	**Patents** *Paten*		
4.	**Specific / Potential Applications** *Spesifik/Potensi aplikasin*		
5.	**Networking & Linkages** *Jaringan & Jalinan*		
6.	**Possible External Research Grants to be Acquired** *Jangkaan Geran Penyelidikan Luar Diperoleh*		

- Kindly provide copies/evidence for Category 1 to 6.

b) Equipment used for this research.
Peralatan yang telah digunakan dalam penyelidikan ini.

Items *Perkara*	Approved Equipment	Approved Requested Equipment	Location
Specialized Equipment Peralatan khusus	1. FLOW CYTOMETRY (BD FacsCanto II) 2. ELISA Plate Reader (PR 5100 Biorad) 3. HAEMATOLOGY COUNTER (Sysmex XS-800i) 4. VORTEX MACHINE (Vortex-2 Genie G 560E) 5. CENTRIFUGE MACHINE (Eppendorf centrifuge 5810 R) 6. INCUBATOR (Memmert)		1. IMMUNOLOGY LABORATORY 2. MICROBIOLOGY LABORATORY
Facility Kemudahan	1. COLD ROOM 2. REFRIGERATOR (Ilshin deep freezer)		1. IMMUNOLOGY LABORATORY

	Infrastructure Infrastruktur	1. RESEARCH LAB 2. DIAGNOSTIC LAB		1. IMMUNOLOGY LABORATORY 3. MICROBIOLOGY LABORATORY

I. BUDGET / *BAJET*

Perbelanjaan :Expenditure

Project Account No.	: 1001 / PSKBP / 8120192
Total Approved Budget	: RM 100 000.00
Total Additional Budget	: RM 16 000.00
Grand Total of Approved Budget	: RM 116 000.00

Yearly Budget Distributed

Year 1	: RM 86 730.00
Year 2	: RM 13 270.00
Year 3	: RM

Additional Budget Approved

Year 1	: RM
Year 2	: RM 16 000.00
Year 3	: RM

Total Expenditure	: RM 100 778.87
Balance	: RM 15 221.13

- **Attached - latest final account statement from Treasury (July 2010)**

Signature of Researcher
Tandatangan Penyelidik

2 May 2011
Date
Tarikh

H.	**COMMENTS OF PTJ'S RESEARCH COMMITTEE** *KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ*

General Comments:
Ulasan Umum:

The grant project successfully completed with two outputs; one publication to Asia-Pacific Journal of Public Health and a MSc. student graduated.

PROFESSOR AHMAD SUKARI HALIM
Chairman of Research Commitee
School of Medical Sciences
Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

Signature and Stamp of Chairperson of PTJ's Evaluation Committee
Tandatangan dan Cop Pengerusi Jawatankuasa Penilaian PTJ

Date : 7/7/14
Tarikh :

Signature and Stamp of Dean/ Director of PTJ
Tandatangan dan Cop Dekan/ Pengarah PTJ

PROFESOR ABDUL AZIZ BABA
Dekan
Pusat Pengajian Sains Perubatan
Kampus Kesihatan
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

Date :
Tarikh :

ABSTRACT OF RESEARCH

Epilepsy is a chronic brain disorder that affects more than 50 million people throughout the whole world. The cause of more than half of the sufferers remains unknown (idiopathic), while the rest may have epilepsy due to certain causes of head injury, stroke, drugs withdrawal or other identifiable problems. The prevalence of this disease increases annually persuading researchers to find out the possible relations to these idiopathic epilepsies.

Previous reports suggested that there could be some kind of association between virus infection and lymphocyte-mediated immunity leading to seizures and therefore this study is conducted 1) to compare mean of lymphocyte subsets between epileptic patients and healthy controls, 2) to compare mean of activated $CD4^+$ and $CD8^+$ T cell subsets between epileptic patients and healthy controls, 3) to determine the sociodemographic factors and lymphocyte subsets in epileptic patients and 4) to observe the associations between IgG, IgM in herpes viruses with lymphocyte subsets in epileptic patients.

A total of 190 subjects were enrolled in this study, 95 subjects for each group of healthy controls and epilepsy patients. Subjects recruited fulfilled the inclusion and exclusion criteria. Whole blood and serum samples were tested by flow cytometry and indirect ELISA technique. Statistical analyses were performed using the SPSS version 12.0.

Results revealed that the number of NK cells ($CD16^+56^+$) were significantly higher in epileptic patients (95%CI: -0.141,-0.002; *p* value: 0.042) while the number of activated T cells ($CD8^+CD25^+$) were significantly lower (95%CI: 0.034, 0.222; *p* value: 0.008) compared to healthy controls. Patient education level (OR: 0.227; 95%CI: 0.127; 0.404; *p* value: 0.001), the number of $CD16^+56^+$ (OR: 8.759; 95%CI: 1.643; 46.685; *p* value: 0.011) and the number of $CD4^+CD25^+$ (OR: 0.229; 95%CI: 0.092 ;0.571; *p* value: 0.002) were the only parameters that were significantly associated with epilepsy. No significant correlation observed between lymphocyte subsets and herpes viruses infection, however Cytomegalovirus (CMV) IgG shows a significant difference in epileptic patients as compared to healthy controls ($p < 0.001$).

This study may suggest that the divergence in NK cells and activated $CD8^+$ T cells ($CD8^+CD25^+$) provides the idea that epilepsy patients has an immune disorder, whereas, previous CMV infection may play a great role in contributing to epilepsy.

ABSTRAK

Epilepsi merupakan sejenis gangguan kronik berlaku dalam otak yang telah menjangkiti lebih dari 50 juta penduduk dunia. Lebih dari separuh penghidap penyakit ini tidak diketahui diketahui puncanya (idiopatik) manakala yang lain mungkin disebabkan oleh kecederaan otak, strok, pengambilan dadah atau sebab-sebab jelas yang lain. Kekerapan kejadian penyakit ini yang bertambah setiap tahun telah mendorong penyelidik untuk mencari segala kaitan kepada punca penyakit epilepsi idiopatik ini.

Kajian terdahulu mencadangkan kemungkinan terdapat kaitan antara jangkitan virus dan imuniti berperantaraan-limfosit yang menjurus kepada sawan, oleh kerana itu, kajian ini dijalankan untuk 1) membandingkan antara purata subset limfosit pesakit epilepsi dan individu kontrol yang sihat, 2) untuk membandingkan purata subset limfosit $CD4^+$ dan $CD8^+$ yang diaktifkan antara pesakit epilepsi dan kontrol yang sihat, 3) untuk menentukan faktor-faktor sosiodemografik dan subset limfosit pesakit epilepsi dan 4) untuk memerhatikan kaitan antara IgG, IgM virus herpes dengan subset-subset limfosit pesakit epilepsi.

Sejumlah 190 subjek di calonkan dalam kajian ini dengan 95 subjek bagi setiap kumpulan kontrol yang sihat dan kumpulan pesakit epilepsi. Subjek yang diambil untuk kajian telah memenuhi kriteria-kriteria penerimaan dan penolakan. Sampel darah dan serum diuji dengan menggunakan kaedah Flowsitometri dan teknik ELISA tidak langsung. Analisis statistik dijalankan menggunakan SPSS versi 12.0.

Keputusan menunjukkan bahawa jumlah sel NK ($CD16^+56^+$) adalah lebih tinggi dalam pesakit epilepsi (95%CI: -0.141,-0.002; nilai *p*: 0.042) manakala jumlah sel T teraktif ($CD8^+CD25^+$) lebih rendah (95%CI: 0.034, 0.222; nilai *p*: 0.008) jika dibandingkan dengan kontrol yang sihat. Tahap pendidikan pesakit (OR: 0.227; 95%CI: 0.127; 0.404; nilai *p*: 0.001), jumlah sel $CD16^+56^+$ (OR: 8.759; 95%CI: 1.643; 46.685; nilai p : 0.011) dan jumlah sel $CD4^+CD25^+$ (OR: 0.229; 95%CI: 0.092; 0.571; nilai p : 0.002) sahaja yang merupakan parameter yang berkait secara signifikan dengan epilepsi. Tiada korelasi yang signifikan dilihat antara subset limfosit dan virus herpes, walaubagaimanapun, IgG terhadap Cytomegalovirus (CMV) telah menunjukkan perbezaan yang ketara antara pesakit epilepsi dan kontrol yang sihat (p <0.001).

Kajian ini mencadangkan bahawa kelainan pada bilangan sel NK dan sel T teraktif ($CD8^+CD25^+$) memberikan idea bahawa dalam pesakit epilepsi berlaku gangguan imun, manakala, sejarah jangkitan terdahulu CMV, mungkin memainkan peranan yang besar dalam menyumbang kepada epilepsi.