UNIVERSITI SAINS MALAYSIA
GERAN PENYELIDIKAN UNIVERSITI
PENYELIDIKAN
LAPORAN AKHIR

CONTRIBUTION OF INCREASED APOPTOSIS IN MONOCYTES AND LYMPHOCYTES IN THE ETIOPATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

PENYELIDIK

PROF. MADYA DR. CHE MARAINA CHE HUSSIN

PENYELIDIK BERSAMA

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DR. NOORSURYANI MOHD ASHARI
DR. WAN SYAMIMEE WAN GHAZALI

2012

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RU GRANT (1001/PPSP/811081)

RESEARCHERS

DR CHE MARAINA CHE HUSSIN

PROF. MADYA DR KAMALIAH MOHD. DAUD

DR NOORSURYANI MOHD ASHARI

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UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT FINAL REPORT
Geran Penyelidikan Universiti Laporan Akhir

A.	**PARTICULARS OF RESEARCH** / *MAKLUMAT PENYELIDIKAN:*
(i)	**Title of Research:** *Tajuk Penyelidikan:* CONTRIBUTION OF INCREASED APOPTOSIS IN MONOCYTES AND LYMPHOCYTES IN THE ETIOPATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE).
(ii)	**Account Number:** *Nombor Akaun:* 1001/PPSP/811081
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader:** *Nama Ketua Penyelidik:* PM DR CHE MARAINA CHE HUSSIN
	Name of Co-Researcher *Nama Penyelidik Bersama:* PM DR KAMALIAH MOHD DAUD DR NOOR SURYANI MOHD ASHARI DR WAN SYAMIMEE WAN GHAZALI
(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 :** 30 MONTHS
Tempoh :

From : JANUARY 2009 **To** : JUNE 2011
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

- Both apoptotic lymphocytes and monocytes was significantly higher in SLE patients compared to normal controls ($p=0.001$ and $p=0.030$) respectively.
- Both apoptotic lymphocytes and monocytes was also significantly higher in active SLE compared to inactive SLE patients ($p<0.042$ and $p<0.028$) respectively.
- According to Pearson's correlation test, apoptotic lymphocyte was significantly correlated with C3(0.007), C4(0.034), TNFα(0.012), anti-dsDNA antibodies(0.029) and anti-C1q anutoantibodies(0.036) but not with C5a, sFas ligand and ACL antibodies.
- Apoptotic monocytes was also significantly correlated with C3(0.001), C4(0.008) and TNFα(0.003).
- The simple linear regression showed no significant relationship between both apoptotic lymphocytes and monocytes with CRP.

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
Systemic lupus erythematosus	Eritematosus lupus sistemik
Apoptosis	Sel mati semulajadi
Monocytes	Monosit
Lymphocytes	Limfosit
Autoimmunity	Autoimuniti
Flowcytometry	Flowsitometri

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	2 Rheumatology International & International Medical journal
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) 7. BIOSAFETY CABINET		IMMUNOLOGY LABORATORY
Facility Kemudahan	1. COLD ROOM 2. REFRIGERATOR (Ilshin deep freezer)		IMMUNOLOGY LABORATORY
Infrastructure Infrastruktur	1. RESEARCH LAB 2. DIAGNOSTIC AB		IMMUNOLOGY LABORATORY

- Please attach appendix if necessary.

I. **BUDGET** / *BAJET*

Total Approved Budget	: RM 174,790.00
Total Additional Budget	: RM
Grand Total of Approved Budget	: RM 174,790.00

Yearly Budget Distributed

Year 1	: RM 88,200.00
Year 2	: RM 86,590.00
Year 3	: RM

Additional Budget Approved

Year 1	: RM
Year 2	: RM
Year 3	: RM

Total Expenditure	: RM 174,678.88
Balance	: RM 111.12

- **Please attach final account statement from Treasury**

H.	**COMMENTS OF PTJ'S RESEARCH COMMITTEE** *KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ*
	General Comments: *Ulasan Umum:* project completed successfully and all objectives achieved. output of project include one published paper and another (second paper) submitted.

ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease involving multiple organ systems and production of a wide array of autoantibodies. The aetiopathogenesis remains unknown but the complex combination of environmental trigger (infection and UV exposure) and genetic factors seems to be involved. Data from human SLE reported an increased apoptosis and defective clearance of apoptotic cells and suggested to have a pathogenic role in development of SLE. Increased apoptosis of circulating and *in vitro* in variety of cells such as monocytes, lymphocytes, neutrophils, as well as cell line was demonstrated in SLE.

Previous reports suggested that the serum factors could induce apoptosis in SLE. Therefore this study is conducted 1) to compare the in vitro mean proportion of apoptosis in monocytes and lymphocytes in active with inactive SLE patients, 2) to compare the in vitro mean proportion of apoptosis in monocytes and lymphocytes in SLE patients with normal controls and 3) to determine the correlation of apoptosis in SLE patients with serum factors.

A total of 248 subjects were enrolled in this study, 124 subjects for each group of healthy controls and SLE patients (62 active SLE and 62 inactive SLE). All the patients fulfilled four or more of the American Rheumarism Association Classification Criteria for SLE (1987).Subjects recruited fulfilled the inclusion and exclusion criteria. Blood was withdrawn into EDTA (1 ml) for full blood count and plain tubes (9 ml) for apoptosis and serum factors analysis. Statistical analyses were performed using the SPSS version 18.0.

Results revealed that both apoptotic lymphocytes and monocytes was significantly higher in SLE patients compared to normal controls ($p=0.001$ and $p=0.030$) respectively. Both apoptotic lymphocytes and monocytes was also significantly higher in active SLE compared to inactive SLE patients ($p<0.042$ and $p<0.028$) respectively. According to Pearson's correlation test, apoptotic lymphocyte was significantly correlated with C3(0.007), C4(0.034), TNFα(0.012), anti-dsDNa antibodies(0.029) and anti-C1q autoantibodies(0.036) but not with C5a, sFas ligand and ACL antibodies. Apoptotic monocytes was also significantly correlated with C3(0.001), C4(0.008) and TNFα(0.003). The simple linear regression showed no significant relationship between both apoptotic lymphocytes and monocytes with CRP ($P>0.05$).

The results of this study suggest that circulating factors in the serum of the SLE patients are responsible for the apoptosis and may play a pathogenic role in SLE.

ABSTRAK

Eritematosus lupus sistemik(SLE) adalah penyakit autoimun yang melibatkan banyak organ dan penghasilan berbagai jenis autoantibodi. Penyebab utama masih tidak diketahui tetapi kombinasi kompleks faktor persekitaran (jangkitana dan pendedahan UV) dan genetik seolah-olah terlibat. Data dari SLE melaporkan bahawa sel mati semulajadi meningkat dan kerosakan penghapusan sel mati dan mencadangkan mempunyai peranan patogen dalam menyumbang kepada SLE. Peningkatan sel mati semulajadi dalam peredaran dan ''in vitro'' dalam pelbagai sel seperti monosit, limfosit, neutrofil dan sel kanser telah ditunjukkan di dalam SLE.

Kajian terdahulu mencadangkan bahawa faktor-faktor serum boleh mendorong kepada sel mati semulajadi dalam SLE. Oleh itu, kajian ini dijalankan adalah 1) untuk membandingkan purata kadar apoptosis di dalam monosit dan limfosit secara in vitro di dalam pesakit SLE yang aktif dan tidak aktif, 2) untuk membandingkan purata kadar sel mati semulajadi di dalam monosit dan limfosit secara in vitro di dalam pesakit SLE dan kontrol normal dan 3) untuk menentukan korelasi sel mati semulajadi dalam pesakit SLE dengan faktor serum.

Sejumlah 248 subjek di calonkan dalam kajian ini dengan 124 subjek bagi setiap kumpulan kontrol yang sihat dan kumpulan pesakit SLE (62 SLE aktif dan 62 SLE tidak aktif). Semua peaskit SLE memenuhi empat atau lebih kriteria pengkelasan SLE Persatuan Rheumatik Amerika. Subjek yang diambil untuk kajian telah memenuhi kriteria-kriteria penerimaan dan penolakan. Sampel darah telah di ambil dan dimasukkan ke dalam tiub EDTA (1 ml) untuk anlysis "Full blood count" dan dalam tiub plain untuk sel mati dan faktor-faktor serum. Analisis statistik dijalankan menggunakan SPSS versi 18.0.

Keputusan menunjukkan bahawa kedua-dua sel mati semulajadi di dalam limfosit dan monosit lebih tinggi di dalam SLE berbanding kontrol ($p=0.001$ and $p=0.030$) secara berturut-turut. Sel mati semulajadi di dalam limfosit dan monosit juga lebih tinggi di dalam SLE yang aktif berbanding SLE yang tidak aktif ($p=0.042$ and $p=0.028$) secara berturut-turut. Menurut kolerasi Pearson, sel mati semulajadi dalam limfosit mempunyai kolerasi dengan C3(0.007), C4(0.034), TNFα(0.012), anti-dsDNA antibodi(0.029) dan anti-C1q autoantibodi(0.036) tetapi tidak dengan C5a, sFas Ligand dan ACL antibodi. Sel mati semulajadi monosit juga mempunyai kolerasi dengan. Regresi linear ringkas menunjukkan tiada hubungan yang signifikan antara kedua-dua sel mati semulajadi dalam limfosit dan monosit dengan CRP($P>0.05$).

Kajian ini mencadangkan bahawa faktor-faktor di dalam serum pesakit SLE bertanggungjawab dalam sel mati semulajadi dan mungkin memainkan peranan patogen dalam menyumbang kepada SLE.