# A STUDY OF MICRO RNA PROFILES IN ISCHAEMIC STROKE AND ITS RISK FACTOR IN DEVELOPMENT OF THE NOVEL BIOMARKER OF YOUNG STROKE PATIENTS

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## Abstract

Recent global stroke surveillance conducted by World Health Organization (WHO) has reported 15 million people suffer from stroke per annum. Approximately one-third of the stroke causes mortality placing it as the second leading cause of death after cardiac arrest. Knowledge pertaining stroke has been valuable and utmost desired in order to deliver effective education, prevention, diagnosis/classification and management. Emergence of microRNA expression level delivering promising biomarker has driven diligent researches in developing novel biomarkers for stroke. MicroRNA is a class of ~22 nucleotide-long non-coding RNAs, which is shown to orchestrate protein expression at posttranscriptional levels, hence earlier detection prior manifestation. Acquiring the-state-of-the-art Real-time PCR technology in RNA quantification, this study was aimed to study the microRNA-145, -214, -222, -223, -23b and -339 present in peripheral blood and its temporal expression profiles of chronic young ischaemic stroke patients; having an insight of the molecular regulation of cerebrovascular maladies origin. Peripheral blood of chronic ischaemic stroke patients (n = 32) aged between 18-49 years, characterized based on WHO clinical criteria which subsequently classified in accordance to TOAST guideline were tested in this study. Subsequent peripheral blood sampling of these patients (n = 11) were performed between 3 to 16 months after the initial sampling in order to study the long-term stroke temporal expression profiles of each microRNAs studied. Peripheral blood form normal volunteers (n = 14) were used as controls. Ribosomal RNA, 18S rRNA expression was used as housekeeping gene. Students' t-Test was employed for statistical calculation. P value of lesser then 0.05 ($p < 0.05$) is considered significant. Results revealed the circulating microRNA-145, -222, -223 and -23b that are implicated in the vascular biology and immune response in young ischaemic stroke patients demonstrated ectopic

expression profile relative to the normal control. The temporal expression profile of circulating microRNA-214, -222, -223 and -23b demonstrated plummet in expression; this suggests recovery and downregulation of microRNAs expression is associated with good outcome following stroke. Comparative analysis of the 6 circulating microRNAs and the subtypes of ischaemic stroke suggested that the microRNA-145, 223, 23b and 339 expression profile in cardioembolic subtype of stroke might differ from the atherosclerotic stroke that comprises of large and small vessel subtypes. In addition, the expression profile of circulating microRNA-23b was also shown to be significantly different between the cardioembolic and undetermined subtypes of ischaemic stroke. Therefore, this study demonstrates the potential of peripheral blood microRNAs to be developed as diagnostic and prognostic biomarkers of cerebral ischaemic stroke.

## Abstrak

Hasil kaji selidik global terkini yang dilangsungkan oleh World Health Organization (WHO) telah melaporkan 15 juta individu mengalami stroke setiap tahun. Daripada itu, lebih kurang satu pertiga daripada kes strok memudaratkan lantas menempatkannya penyebab kedua kematian sejurus serangan jantung. Namun, pengetahuan mengenai strok penting dan diperlukan demi penyampaian pendidikan efektif, pencegahan, diagnostik/klasifikasi dan penyelengaraan. Kemunculan taraf ekspersi mikroRNA sebagai biomarker telah mencetus kegigihan para penyelidik dalam menempuh penemuan biomarker ungul bagi strok. MikroRNA merupakan kumpulan RNA tidak mengkod yang terdiri daripada ~22 rantaian nukeotida yang mampu mengolah ekspersi protein pada tahap pasca-transkripsi, yakni membolehkan pengesana awal sebelum menifistasi. Dengan penggunaan teknologi pengkuantitian Real-time PCR, kajian ini dijalankan dengan tujuan menkaji mikroRNA-145, -214, -222, -223, -23b dan -339 yang hadir dalam darah periferi dan profil ekspresi temporalnya dalam pesakit kronik muda strok iskemia; demi penghayatan regulasi molekul yang berpunca daripada masalah cerebrovaskular. Darah periferi pesakit kronik strok iskemia (n = 32) yang berumur diantara 18-49 tahun, diklasifikasi dengan merujuk kepada kriteria klinikal WHO yang selanjutnya diklasifikasi mengikut tatacara TOAST telah digunakan bagi menjalankan kajian ini. Pensampelan darah periferi lanjutan (n = 11) dijalankan diantara 3 hingga 16 bulan selapas pensampelan pertama dilakukan bagi menkaji profil ekspresi temporal setiam mikroRNA yang dikaji. Darah periferi yang diperolehi daripada sukarelawan normal (n = 11) digunakan sebagai piawai. Ekspresi RNA ribosomal, 18S rRNA dirujuk sebagai gen housekeeping. Students' t-Test digunakan dalam penghitungan statistik. Nilai p yang kurang daripada 0.05 ($p < 0.05$) dikira signifikan. Keputusan menunjukkan mikroRNA-145, -222, -223 dan -23b yang terdapat

dalam pengaliran darah yang menmunyai implikasi terhadap biologi vaskular dan tindak balas immune dalam pesakit muda stroke iskemia menunjukkan profail ekspresi ectopic relatif kepada piawai normal. Profail ekspresi temporal mikroRNA-214, -222, -223, dan -23b yang terdapat dalam pengaliran darah menunjukkan penjunaman ekspresi. Hasil analisis 6 mikroRNA yang di kaji melalui perbandingan yang dibuat terhadap sub-jenis strok iskemia telah mecadangkan bahawa profile ekspresi mikroRNA-145, -222, -23b dan -339 pada sub-jenis cardioembolic mungkin berbeza daripada atherosclerotic strok yang terdiri daripada sub-jenis large vessel dan small vessel. Tambahan pula, profile ekspresi mikroRNA-23b dari darah periferi juga menunjukkan perbezaan yang signifikan diantara sub-jenis cardioembolic dan sub-jenis strok iskemia yang tidak dikenalpasti. Ini mencadangkan pemulihan dan pengurangan regulasi ekspresi microRNA dikaitkan dengan hasil baik selanjut strok. Dengan ini, hasil kajian ini telah menyerlahkan potensi microRNA darah periferi dalam penghasilan biomarker prognostik strok iskemia cerebral.

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# Contents

# List of Figures

# List of Tables

## List of Appendixes

## Abbreviations

| | |
|---|---|
| - | negative/minus |
| % | percent |
| + | positive/plus |
| ∝ | infinity |
| ® | registered |
| °C | degree Celsius |
| Δ | delta |
| µl | micro liter |
| $A_{260}$ | absorbance at 260 nano meter wave lengths |
| $A_{280}$ | absorbance at 280 nano meter wave lengths |
| Ago | argonaute |
| AHA | the American heart association |
| BP | blood pressure |
| bp | base pair |
| cDNA | complimentary ribonucliec acid |
| $C_T$ | cycle threshold |
| CT | computed topography |
| DGCR8 | DiGeorge syndrome critical region 8 gene |
| dNTP | deoxyriboneucleotide triphosphate |
| ECG | electrocardiography |
| Elk-1 | E twenty-six-like transcription factor 1 |
| eNOS | endothelial nitric oxide synthase |
| EPC | endothelial progenitor cells |
| FRET | foster resonance energy transfer through space |
| g | gram |
| G1 phase | gap 1 phase |
| $G_0$ | gene ontology |
| has | *Homo sapien* |
| HbA1c | hemoglobin A1c |
| HbF | fetal hemoglobin |
| HDL | high-density lipoprotein |

| | |
|---|---|
| HUVEC | human umbilical vein endothelial cells |
| ICAM-1 | intracellular cell adhesion molecule-1 |
| KEGG | Kyoto encyclopedia of genes and genomes |
| KLF | kruppel-like factor |
| LMO2 | LIM-only protein 2 |
| M phase | Mitotic phase |
| MADS | MCM1-agamous-deficient serum response factor |
| Mef2c | myocyte enhancer factor 2c |
| MGB | minor groove binder |
| MHC | myosin heavy chain |
| miR | micro ribonucleic acid |
| ml | mili liter |
| mM/l | mili molar per liter |
| MMAC1 | mutated in multiple advance cancers |
| mmHg | mili meter mercury |
| MMP9 | matrix metallopeptidase 9 |
| MRI | magnetic resonance imaging |
| mRNA | messenger ribonucleic acid |
| NFQ | nonflorescent quencher |
| ng | nano gram |
| nm | nano meter |
| NSE | neuron specific enclose |
| OD | optical density |
| p | p value |
| p53 | protein 53 |
| PAGE | polyacrylamide gel electrophoresis |
| PCR | polymerase chain reaction |
| PDE4D | phosphodiesterase 4D |
| PI3K | phosphoinositide 3 kinase |
| pre | precursor |
| pri | primary |
| PTEN | phosphate and tensin homolog protein |

| | |
|---|---|
| RISC | ribonucleic acid induced silencing complex |
| RNA | ribonucleic acid |
| RNAi | ribonucleic acid interference |
| rRNA | ribosomal ribonucleic acid |
| RT-PCR | reverse transcription polymerase chain reaction |
| S | svedberg |
| S phase | Synthesis phase |
| S.E.M. | standard error mean |
| S100B | S100 calcium binding protein B |
| SCF | stem cell factor |
| SM | smooth muscle |
| SMC | smooth muscle cell |
| SRF | serum response factor |
| TIA | transient ischaemic attack |
| ™ | trade mark |
| TOAST | trial of ORG 10172 in acute stroke treatment |
| tRNA | transfer ribonucleic acid |
| UMMC | University Malaya Medical Centre |
| UTR | untranslated region |
| UV | ultra-violet |
| V | volt |
| VCAM-1 | vascular cell adhesion protein-1 |
| VSMC | vascular smooth muscle cell |
| WHO | world health organization |