

**GENE EXPRESSION PROFILING FOR POSTOPERATIVE  
COGNITIVE DYSFUNCTION (POCD) AMONG HIGH RISK  
CORONARY ARTERY BYPASS GRAFT (CABG) PATIENTS**

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**UNIVERSITI SAINS ISLAM MALAYSIA**

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CORONARY ARTERY BYPASS GRAFT (CABG) PATIENTS**

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## ABSTRAK

Penurunan fungsi kognitif selepas pembedahan (*postoperative cognitive dysfunction, POCD*) ialah komplikasi yang tidak diingini serta memberi impak yang signifikan kepada kualiti kehidupan pesakit. POCD boleh dicirikan dengan kecelaruan mental, keresahan, perubahan personaliti dan kecelaruan memori. Kajian ini bertujuan untuk mengenalpasti ekspresi gen terbeza (*differentially expressed genes, DEGs*) dalam kalangan pesakit pintasan koronari arteri (*coronary artery bypass graft, CABG*) berisiko tinggi yang menghidap POCD dan tahap penanda bio protin yang terlibat dengan ekspresi gen tersebut. Empat puluh enam pesakit yang dijadualkan untuk prosedur CABG berisiko tinggi dipilih berdasarkan kriteria inklusi dan eksklusi yang ketat. Penilaian kognitif dilakukan satu hari sebelum dan 7 hari selepas prosedur untuk pengklasifikasian POCD. Bagi analisis DEGs menggunakan teknik susunan mikro, sampel asid ribonukliek (*ribonucleic acid, RNA*) diekstrak daripada darah pesakit pada 1 hari sebelum dan 3 hari selepas prosedur. Sebanyak 16 sampel daripada 46 pesakit dipilih untuk analisis tersebut. Kemudian, penentuan tahap penanda bio protin daripada DEGs dilakukan menggunakan ujian enzim-pengerap immuno (*ELISA*). Ujian sampel-T tidak bersandar dan sampel-T bersandar dijalankan untuk melihat perbezaan antara kumpulan. Berdasarkan analisis sebelum dan selepas pembedahan bagi pesakit POCD, hasil kajian mendapati 5 gen ekspresi terbeza. Gen pengawalan menurun ialah KIR3DL2, KIR2DS3, KIR2DS2 yang terlibat dalam respon pengawalan imun, dan LIM2, gen yang terlibat dalam penghasilan katarak. Manakala gen pengawalan meningkat ialah ERFE yang terlibat dalam metabolisme zat besi. Bagi analisis selepas pembedahan bagi pesakit POCD dan bukan POCD, terdapat satu gen pengawalan menurun iaitu BTNL3, yang terlibat dalam pengawalan imun. Kepekatan protin eritroferon, ERFE didapati lebih tinggi secara signifikan pada pesakit POCD berbanding pesakit bukan POCD ( $p < 0.05$ ). Lengkuk *Receiver Operating Characteristic (ROC)* dihasilkan dan mendapati ERFE meramal kejadian POCD secara sederhana dengan nilai takat ditetapkan pada 0.761, dengan spesifikasi dan sensitiviti pada 80% dan 50% (luas bawah lengkung: 0.685, 95% CI: 0.564 hingga 0.802,  $p < 0.05$ ). Kesimpulannya, penglibatan pengawalan imun dan metabolisme zat besi adalah gen berpotensi terlibat dalam kejadian POCD. Kepekatan gen ERFE dalam plasma berpotensi menjadi penanda bio bagi meramal POCD. Kajian ini menjadi perintis untuk pemahaman POCD di tahap molekular.

## ABSTRACT

Postoperative cognitive dysfunction (POCD) is the undesirable complication following surgery that significantly impacts patients' quality of life. The outcome of POCD is characterized by mental disorders, anxiety, personality changes and impaired memory. This study aimed to identify the differentially expressed genes (DEGs) in high-risk CABG patients with POCD and the level of the protein biomarker that correlates with the expressed gene. Forty-six patients scheduled for high-risk CABG procedures were selected based on inclusion and exclusion criteria. Cognitive assessments were done one day before and seven days after surgery to classify POCD. For DEGs analysis using microarray, ribonucleic acid (RNA) samples were collected from patients' blood one day preoperative and 3 days postoperatively. A total of 16 samples from the 46 patients were selected for microarray analysis. Further, an enzyme-linked immunosorbent assay (ELISA) was conducted to determine the level of protein biomarkers related to the DEGs. Independent T-test and paired T-test were performed to compare the differences between groups. Based on pre and postoperative analysis of POCD patients, the findings revealed 5 differentially expressed genes. The downregulated genes were KIR3DL2, KIR2DS3, and KIR2DS2, which are involved in immunoregulatory responses, and LIM2, a gene involved in cataractogenesis. Whilst the upregulated gene was ERF, that is involved in iron metabolisms. For postoperative analysis of POCD and non-POCD patients, one gene was downregulated, which is BTNL3, that involved is in immunoregulation. A significantly higher concentration of erythroferrone protein, ERF, was found in POCD patients' plasma than in non-POCD patients ( $p < 0.05$ ). Receiver Operating Characteristic (ROC) curve was generated and revealed that ERF has a moderate performance in predicting POCD with the threshold set at 0.761 with specificity and sensitivity at 80% and 50% (AUC:0.685, 95% CI: 0.564 to 0.802,  $p < 0.05$ ). In conclusion, immunoregulatory and iron metabolism genes are potential genes involved in POCD development. ERF concentration in plasma may potentially be used as a biomarker for clinicians to predict POCD. This study serves as a pioneer in understanding POCD at the molecular level.

## الخلاصة

يعد الخلل المعرفي بعد العملية الجراحية (POCD) من المضاعفات غير المرغوب فيها بعد الجراحة والتي تؤثر بشكل كبير على نوعية حياة المرضى. تتميز نتائج POCD بالاضطرابات العقلية والقلق وتغيرات الشخصية وضعف الذاكرة. تهدف هذه الدراسة إلى تحديد الجينات المعبر عنها تفاضلياً (DEGs) لدى مرضى CABG المعرضين للخطر والذين يعانون من POCD ومستوى العلامة الحيوية للبروتين التي ترتبط بالجين المعبر عنه. تم اختيار ستة وأربعين مريضاً من المقرر إجراؤهم لإجراءات تحويل مسار الشريان التاجي عالية الخطورة بناءً على معايير التضمين والاستبعاد. تم إجراء التقييمات المعرفية قبل يوم واحد وبعد الجراحة بسبعة أيام لتصنيف POCD. لتحليل DEGs باستخدام ميكروأري، تم جمع عينات من حمض الريبونوكلييك (RNA) من دم المرضى قبل يوم واحد من الجراحة وبعد 3 أيام من العمل الجراحي. تم اختيار ما مجموعه 16 عينة من 46 مريضاً للتحليل. علاوة على ذلك، تم إجراء مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لتحديد مستوى المؤشرات الحيوية للبروتين المرتبطة بـ DEGs. تم إجراء اختبار T المستقل واختبار T المقترن لمقارنة الاختلافات بين المجموعات. استناداً إلى التحليل قبل وبعد العملية الجراحية لمرضى POCD، كشفت النتائج عن 5 جينات معبر عنها تفاضلياً. الجينات منخفضة التنظيم هي KIR3DL2، وKIR2DS3، وKIR2DS2، والتي تشارك في الاستجابات التنظيمية المناعية، وLIM2، وهو الجين المشارك في تكوين إعتام عدسة العين. في حين أن الجين المنتظم هو ERFE، الذي يشارك في استقلاب الحديد. بالنسبة لتحليل ما بعد الجراحة لمرضى POCD والمرضى غير POCD، تم تقليل تنظيم جين واحد، وهو BTNL3، الذي يشارك في التنظيم المناعي. تم العثور على تركيز أعلى بكثير من بروتين الإريثروفيرون، ERFE، في بلازما مرضى POCD مقارنة بالمرضى غير المصابين بـ (( $0.05 < p < 0.05$ ). تم إنشاء منحنى خصائص تشغيل المستقبل (ROC) وكشف أن ERFE يتمتع بأداء معتدل في التنبؤ بـ POCD مع تحديد العتبة عند 0.761 مع خصوصية وحساسية عند 80% و50% (AUC:0.685، 95% CI: 0.564 إلى 0.802،  $p < 0.05$ ). (في الختام، الجينات المناعية واستقلاب الحديد هي الجينات المحتملة المشاركة في تطوير POCD. من المحتمل أن يتم استخدام تركيز ERFE في البلازما كمؤشر حيوي للأطباء للتنبؤ بـ POCD. تعد هذه الدراسة رائدة في فهم POCD على المستوى الجزيئي.

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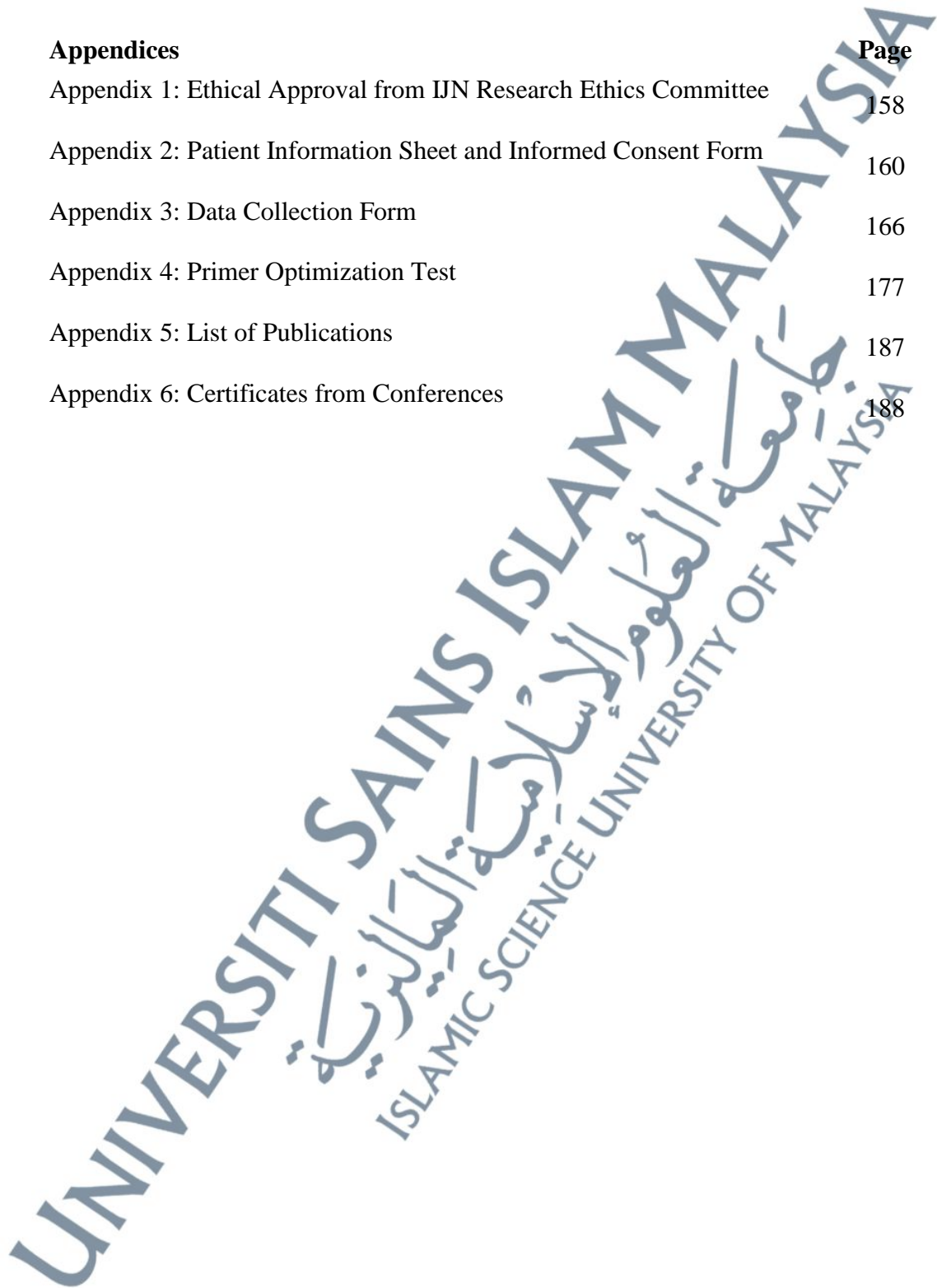
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## LIST OF ABBREVIATIONS

APOE-ε4	apolipoprotein epsilon 4
CABG	coronary artery bypass graft
CeOx	cerebral oximetry
CPB	cardiopulmonary bypass
CT	cycle threshold
DEG	differentially expressed genes
eGFR	estimated glomerular filtration rate
MMSE	mini-mental state examination
PCA	principal component analysis
POCD	postoperative cognitive dysfunction
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
rScO2	regional cerebral oxygen saturation
KIR	killer cell immunoglobulin like receptor
ERFE	erythroferrone
LIM2	lens intrinsic membrane protein 2
BTNL3	butyrophilin like 3
MORN4	MORN repeat containing 4
CCL23	C-C motif chemokine ligand 23
GPR37L1	G protein-coupled receptor 37 like 1
ADAMTS2	ADAM metallopeptidase with thrombospondin type 1 motif 2
DCANP1	Dendritic cell associated nuclear protein