MicroRNA (miRNA) PROFILE IN ACUTE MYOCARDIAL INFARCTION (AMI) OF YOUNG ADULTS IN KUANTAN, PAHANG

BY

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ABSTRACT

Acute myocardial infarction (AMI) is a severe form of coronary heart disease where Malaysians are getting AMI at younger age compared to well-developed countries. MicroRNAs (miRNAs) are implicated in AMI pathogenesis, but no study looked at their profiling or involvement in young population. The present study aims to profile the miRNAs expressions in healthy controls (aged 18 to 45 years), young AMI (YAMI) (aged ≤ 45 years), and mature AMI (MAMI) (aged ≥ 46 years) patients with matching criteria and to determine the effect of the dysregulated miRNAs on the target mRNAs as well as the pathways involve in the pathogenesis of AMI. This study was conducted on twenty Malay males for each group in Kuantan, Pahang. Total RNA was extracted from plasma and the miRNA expression profiling was carried out on the BGISEQ500 SE50 sequencing platform with BGI sequencing libraries. The sequence data were analyzed using Gene Ontology (GO) to determine the role of the differentially expressed genes, followed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis for identification of the biological pathways in YAMI against MAMI. The top six dysregulated miRNAs identified during sequencing were validated using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) between the groups. ANOVA and unpaired T-test were used to analyze the differences of miRNAs and gene expression between the three groups. This study revealed that majority AMI patients were smokers, where YAMI patients had higher BMI, SBP, DBP and TG while MAMI patients had higher FBG than the rest of the group. A total of 1599 miRNAs were differentially expressed in AMI (YAMI and MAMI) patients compared to healthy controls, where 1288 were upregulated and 311 were downregulated (FDR ≤ 0.001). However, when YAMI patients were compared to MAMI patients, 1497 miRNAs were found to be dysregulated, of which 1090 miRNAs were upregulated, and 407 miRNAs were downregulated (FDR ≤ 0.001). The top ten upregulated miRNAs were miR-552, miR-4446-3p, miR-432-5p, miR-548j-5p, miR-219, miR-982, miR-181a-2-3p, miR-654-5p, miR-58 and miR-548k; while the top ten downregulated were miR-16-5p, miR-1064, miR-431-5p, miR-790 miR-1177, miR-201, miR-105, miR-518, miR-419 and miR-1103. This study also discovered ten novel miRNAs: miR-4446-3p, miR-982, miR-58, miR-548k, miR-1064, miR-790, miR-1177, miR-201, miR-419, and miR-1103. The validation of the top six dysregulated miRNAs between YAMI and MAMI patients revealed the upregulation of miR-423-5p by 2.08-fold ($p = 0.040$) and downregulation of miR-431-5p by 33.90-fold ($p = 0.034$), and miR-378a-5p by 34.61-fold ($p = 0.040$). For these 1497 differentially expressed miRNAs, 34,195 target genes were predicted by GO analysis. The functional analysis demonstrated 11,199 GO terms found to be involved in biological processes, 12,012 in cellular components, and 10,984 in molecular functions were significantly enriched ($p < 0.05$). The target genes that were mapped to the signal transduction pathway in KEGG revealed 346 classes were enriched. In conclusion, miRNAs are differentially expressed between young and mature AMI, ten of which are novel. Three biological pathways, ascorbate and aldarate metabolism, collecting duct acid secretion and glycosaminoglycans biosynthesis – heparin sulfate/heparin were identified but their involvements in the regulatory mechanisms on gene expression in Young AMI need further evaluation.

ملخص البحث

احتشاء العضلة القلبية الحاد (Acute myocardial infarction, AMI) هو مرض قلبي حاد من أمراض القلب التاجية، يصاب به الماليزيون في سن أصغر مقارنة بالدول المتقدمة. الحمض النووي الريبوزي الدقيق (miRNAs) يساهم بشكل رئيسي في التسبب بهذا المرض، ولا توجد دراسة سابقة قامت بتحديد دوره أو دراسة سماته في فئة الشباب. استهدفت الدراسة الحالية تحديد سمات الmiRNAs وتعبيراته الجينية في المجموعات التالية: الأصحاء كمجموعة ضابطة (18–45 عاماً)، ومجموعتين من مرضى الAMI وهي، مجموعة المرضى الشباب YAMI (≤ 45 عاماً) ومجموعة المرضى الكبارMAMI (≥ 46 عاماً)، المرضى ذو المعايير المطابقة تم دراستهم لتحديد تأثير خلل التنظيم في الmiRNAs على الحمض المستهدف من الmRNA و كذلك المسارات المشاركة في التسبب بالمرض. أجريت هذه الدراسة على 20 رجلاً في كل مجموعة، في مدينة كوانتن بإقليم بهانج. أُستخلص حمض الرنا من البلازما وحُددت سمات التعبير الجيني لـ miRNAs على منصة التسلسل BGISEQ500 SE50 وعبر مكتبة بيانات التسلسل. أُجري تحليل بيانات التسلسل باستخدام الأونتولوجيا الجينية (Gene Ontology, GO) لتحديد دور الجينات، متبوعًا بتحليل موسوعة كيوتو للجينات والجينوم الإثرائي لتحديد المسارات البيولوجية في مجموعة الYAMI مقابل مجموعة الMAMI . تم التحقق من صحة أفضل ستة miRNAs ذات خلل في التعبير الجيني وتحديدها أثناء النسخ باستعمال تفاعل البوليمراز المتسلسل الكمي للنسخ العكسي بين المجموعات. واستخدم إحصاء تحليل التباين واختبار "تي" غير المقيد لتحليل الاختلافات في miRNAs والتعبير الجيني بين المجموعات الثلاث. كشفت هذه الدراسة أن غالبية مرضى AMI كانوا مدخنين، حيث كان مؤشر كتلة الجسم أعلى لدى YAMI، وضغط الدم الانقباضي والانبساطي عالي، وارتفاع في مستوى دهن ثلاثي الجليسريد. بينما الMAMI كان لديهم معدل سكر الصيام أعلى في الدم مقارنة ببقية المجموعات. تم التعبير جينياً عن إجمالي 1599 حمضاً من الmiRNAs بشكل تفاضلي في مرضى الAMI (YAMI وMAMI) مقارنةً بالمجموعة الضابطة، حيث كان التعبير بالتنظيم الرفعي 1288 وكان التعبير بالتنظيم التخفيضي 311 (معدل الاكتشاف الخاطئ ≥ 0.001). ومع ذلك، عند مقارنة المرضى الYAMI بالMAMI، تم العثور على 1497 حمض من miRNAs غير منتظم التعبير، منها كان التعبير بالتنظيم الرفعي 1090 حمضاً، و 407 حمضاً كان التعبير بالتنظيم التخفيضي (معدل الاكتشاف الخاطئ ≥ 0.001).ال miRNAs المنظمة رفعيا وفي المراتب العشر الأولى كانت: miR-552, miR-4446-3p, miR-432-5p, miR-548j-5p, miR-219, miR-982, miR-181a-2-3p, miR-654-5p, miR-58 and miR-548k، بينما العشرة الأحماض الأخرى من miRNAs المعبرة بالتنظيم التخفيضي هي: miR-16-5p, miR-1064, miR-431-5p, miR-790, miR-1177, miR-miR-201, miR-105, miR-518, miR-419 and miR-1103. اكتشفت هذه الدراسة أيضاً عشرة أحماض من miRNAs وهي: miR-4446-3p, miR-982, miR-58, miR-548k, miR-11031064, miR-790, miR-miR-1177, miR-201, miR-419, and miR-. أكثر ستة أحماض ذات فعالية عالية من الmiRNAs ذات خلل تعبيري بين المرضى YAMI وMAMI كانت بالتنظيم الرفعي لـ miR-423-5p بمقدار 2.08 طي (p= 0.04)، أما بالتنظيم التخفيضي فكانت لـ miR-431-5p بمقدار 33.9 طي (p= 0.034)، و miR-378a-5pبمقدار 34.61 طي (p= 0.04). بالنسبة إلى هذه الـ1497 حمضاً من miRNAs المعبر عنها تفاضلياً، تم توقع استهداف 34195 جيناً بواسطة تحليل الGO. أظهر التحليل الوظيفي أن 11199 مصطلحاً من مصطلحات GO وجدت أنها مشاركة في العمليات البيولوجية، و 12012 في المكونات الخلوية، و10984 في الوظائف الجزيئية كانت غنية بشكل أكبر ($p < 0.05$). كشفت الجينات المستهدفة التي تم تحديدها لمسار تحويل الإشارة في موسوعة كيوتو للجينات والجينوم أنه تم إثراء 346 فئة. اختصاراً، التعبير الجيني عن miRNAs عبرت بشكل تفاضلي بين مرضى الYAMI والMAMI، عشرة منها جديدة. تم تحديد ثلاثة مسارات بيولوجية وهي: أيض الأسكوربات والألدارات، وإفراز حمض القناة، والتخليق الحيوي للجليكوز أمينو جليكان – كبريتات الهيبارين/الهيبارين، ولكن مشاركتها في الآليات التنظيمية للتعبير الجيني في مجموعة المرضى الشباب تحتاج إلى مزيد من الدراسة. تم تحديد ثلاثة مسارات بيولوجية، أيض الأسكوربات والألدارات، وإفراز حمض القناة، والتخليق الحيوي للجليكوز أمينو جليكان – كبريتات الهيبارين/الهيبارين، لكن مشاركتها في الآليات التنظيمية للتعبير الجيني في مجموعة المرضى الشباب تحتاج إلى مزيد من الدراسة.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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This thesis is dedicated to my husband and my mother for their support, encouragement and unconditional love.

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ACS	Acute coronary syndrome
ACTB	Actin B
ADAM10	A disintegrin and metalloproteinase 10
ADP	Adenosine diphosphate
AGO	Argonaut
Ago 2	Argonaut 2
AIM2	Absent in melanoma 2
AKI	Acute kidney injury
AMI	Acute myocardial infarction
AMPK	Adenosine monophosphate-activated protein kinase
ATG4B	Autophagy-related 4B
ATG7	Autophagy-related 7
ATP	Adenosine triphosphate
AUC	Area under the curve
BCL-2	B-cell lymphoma 2
BGI	Beijing Genome Institute
BMI	Body mass index
BNIP3	BCL-2/adenovirus E1B 19 k-Da protein-interacting protein 3
C5ARI	Complement C5A receptor inhibitor
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CD40	Cluster of differentiation 40
CD40L	Cluster of differentiation 40 ligand
CDC	Center for Disease Control
cDNA	Complementary deoxyribonucleic acid
CHD	Coronary heart disease
circANXA2	Circular RNA ANXA2
cTn	Cardiac troponin
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEG	Differentially expressed gene
DGCR8	DiGeorge syndrome critical region 8
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphate
dsRNA	Double-stranded RNA

DUSP-JNK1/2	Dual-specificity protein phosphatase 1-c-jun N-terminal kinase 1 or 2
ECG	Electrocardiogram
ECM	Extracellular matric
ED	Emergency Department
EDTA	Ethylenediamine tetraacetic acid
FBG	Fasting blood glucose
FOLR3	Folate receptor 3
G6P-DH	Glucose-6-phosphate dehydrogenase
GAG	Glycosaminoglycans
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gDNA	Genomic deoxyribonucleic acid
GDP	Gross domestic product
GK	Glycerol kinase
GMR	Glutamate metabotropic receptor
GO	Gene Ontology
GPO	Glycerol phosphate oxidase
GRM4	Glutamate metabotropic receptor 4
GZMB	Granzyme B
H/R	Hypoxia/reperfusion
H_2O	Water
H_2O_2	Hydrogen peroxide
H9c2	Clonal cell or cell line derived from rat heart tissue
HDL	High-density lipoprotein
HIF-1α	Hypoxia inducible factor 1 alpha
HIPK3	Homeodomain-interacting protein kinase 3
HK	Hexokinase
I/R	Ischaemia/reperfusion
IC	Intercalated cell
IFN-γ	Interferon-gamma
IHD	Ischaemic heart disease
IIUM	International Islamic University Malaysia
IL-1β	Interleukin 1 beta
IL-29	Interleukin 29
IQR	Interquartile range
KEGG	Kyoto Encyclopedia of Genes and Genomes
KKM	Kementerian Kesihatan Malaysia
KLF2	Kruppel-like factor 2
LBBB	Left bundle branch block

LDL	Low-density lipoprotein
LRR	Leucine -rich repeat
LVH	Left ventricular hypertrophy
MADB	Bis dimethylaniline disodium salt
MARCH6	Membrane-associated ring-CH finger protein 6
Mg^{2+}	Magnesium ion
MI	Myocardial infarction
miRNA	MicroRNA
MLKL	Mixed lineage kinase domain-like
MMPs	Matrix metalloproteinases
MOH	Ministry of Health
MREC	Medical Research Ethical Committee
mRNA	Messenger RNA
MSCs	Mesenchymal stem cells
MYBL2	MYB proto-oncogene like 2
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydride
NCVD-PCI	National Cardiovascular Disease – Percutaneous Intervention
NEMO	Nuclear factor-kappa B essential modulator
NF-κB	Nuclear factor kappa B
NF1B	Nuclear factor 1B
NLRP3	Nucleotide-binding domain (NOD-), leucine-rich repeat (LRR), pyrin domain-containg-3
NOD	Nucleotide-binding and oligomerization domain
NOS	Nitric oxide synthase
NSTEMI	Non-ST elevation myocardial infarction
P53 or TP53	Tumour protein 53
PCI	Percutaneous intervention
PCSK9	Proprotein convertase subtilisin kexin 9
piRNA	Piwi-interacting ribonucleic acid
Pre-miRNA	Precursor miRNA
Pri-miRNA	Primary miRNA
PTCH1	Protein patched homolog 1
QC	Quality control
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
qRT-PCR	Quantitative reverse-transcription polymerase chain reactions
RAB22A	Ras-related protein Rab-22A
RBBB	Right bundle branch block

RBP	RNA binding protein
RIPK	Receptor-interacting serine/threonine-protein kinase
RISC	RNA induced silencing complex
RNA	Ribonucleic acid
RNAi	RNA interference
SASMEC	Sultan Ahmad Shah Medical Center
SBP	Systolic blood pressure
SCN4A	Sodium voltage-gated channel type 4 alpha
SD	Standard deviation
SDF	Stromal cell-derived factor
SELT	Selenoprotein T
shRNA	Short hairpin RNA
siRNA	Small interfering ribonucleic acid
SIRT3	Sirtuin 3
SMCs	Smooth muscle cells
snoRNA	Small nucleolar ribonucleic acid
SPSS	Statistical Package for Social Sciences
sRNA-seq	Small RNA sequencing
STEMI	ST elevation myocardial infarction
TAK	TGF-β activated kinase 1
TC	Total cholesterol
TEH	Total health expenditure
TG	Triglyceride
TGF-β	Transforming growth factor beta
TLR4	Toll-like receptor 4
TRIM55	Tripartite motif-containing protein 55
UDP-G	Uridine diphosphate-glucose
UMI	Unique Molecular Identifier
URL	Upper Reference Limit
UTR	Untranslated region
VCMCs	Vascular smooth muscle cells
VDR	Vitamin D receptor
VF	Ventricular fibrillation
WHO	World Health Organization
ZEB1	Zinc finger E-box binding homeobox 1
µL	microliter

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION

Acute myocardial infarction (AMI) is the lethal manifestation of coronary heart disease (CHD), also known as ischaemic heart disease (IHD), with high morbidity and mortality (Roth et al., 2017; WHO, 2021b). It occurs when there is an injury to a part of the heart muscle tissue due to lack of oxygenation, which is usually caused by a partial or total blockage of the coronary blood flow (Thygesen et al., 2018). AMI is the leading cause of death globally and predicted to remain so for the next 20 years (Roth et al., 2017). According to the World Health Organization (WHO), in 2019, 17.9 million people died from cardiovascular disease (CVD), which accounted for 32% of all global deaths, and of these deaths, 85% were due to AMI and stroke (WHO, 2021b). About three fourth of deaths occurred in low- and middle-income countries (WHO, 2021b). This makes CHD, particularly AMI, a serious major public health issue around the world.

In Malaysia, AMI is also the leading cause death. According to the Department of Statistics Malaysia, IHD accounted for 17% of 109,155 medically certified deaths in 2020 (Department of Statistics Malaysia, 2021). In addition to its mortality burden, IHD is also the leading cause of morbidity and loss of quality of life and exerts heavy economic costs with yearly increment in total health expenditure. In 2018, the Malaysian government spent RM60,339 million on total expenditure on health (TEH), which is equivalent to 4.17% of gross domestic product (GDP), rising to RM64,306 million, equivalent to 4.26% of GDP in 2019 (National Health Accounts Malaysia, 2021). With the continuous rise of prevalence and incidence of IHD, the Malaysian Government may need to bear further financial strain on the health care expenditure.

In Asia, particularly Malaysia, people are getting AMI at younger age compared to well-developed countries with the age range of 55.9 to 59.5 years in 2006 to 2010, and 55.0 to 59.3 years in 2012 to 2016 versus 63.4 to 68 years in well-developed countries (Lee et al., 2021; Lu & Nordin, 2013). According to the Malaysian National

Cardiovascular Disease-Percutaneous Coronary Intervention (NCVD-PCI) database, between 2007 to 2009, the prevalence of young AMI under the age of 45 years old is about 16% (Zuhdi et al., 2013) while between 2017 and 2018, the prevalence of AMI under the age of 50 is approximately 23% (Wan Ahmad, 2021). The consequences of AMI can be devastating particularly at "young" age due to the huge impact on patient's psychology, ability to work and socioeconomic burden. Besides, as this young patient may be the main income provider of the family, the repercussion following AMI can also affect multiple dependents.

One of the major factors for developing AMI in this young population is genetic predisposition. A family history of IHD is considered as one of the most relevant risk factors for developing early onset of AMI as positive family history was reported to be higher in young AMI than older AMI patients (Ambroziak et al., 2020; Ge et al., 2017; Lei & Bin, 2019; Venkatason et al., 2019). Therefore, a deeper molecular understanding on the pathological processes of AMI is crucial for both basic cardiovascular and clinical research. Since the study of IHD in young population is important in the era of preventive cardiology, this knowledge is also vital in developing the framework in primary and secondary prevention in the future.

MicroRNAs (miRNAs) are short, single stranded, noncoding RNAs that regulate gene expression post-transcriptionally by binding to the untranslated region (UTR) of the target messenger RNA (mRNA) (Wang et al., 2015). In AMI, miRNAs might affect the atherogenesis, a precursor for AMI by affecting the genes that regulate endothelial stability and atherosclerotic plaque destabilization. miRNAs might also affect the genes involved in the pathogenic pathway of AMI including apoptosis, necrosis, and autophagy. However, information on these theoretical roles of miRNA in young AMI is scarce. Therefore, it is important to dissect further miRNAs involvement in the pathogenesis of AMI in this young population.

Specific miRNAs are postulated to be involved in various stages of AMI pathogenesis in cell culture and animal studies (Ge et al., 2019; Guo et al., 2020; Han, Chen, Su, Zheng, Chen, Sun, Wu, Jiang, Xu, & Yang, 2019; Hao et al., 2020; Huang et al., 2020; Huangfu et al., 2020; Li et al., 2019b; Shi et al., 2020; Shin, Choi, Moon, Lee,

Park, Lee, Seo, Han, Lim, & Lee, 2019; Wang et al., 2020; Zhang et al., 2019a, 2019b). However, their complex regulatory mechanisms have not been completely understood (Schulte et al., 2017b). Though there are few studies in human looking at the involvement of these miRNAs in AMI but none of them studied on young AMI patients. There is a possibility that different miRNAs may be involved in the pathogenesis of AMI in this young population. Understanding the pathogenesis of AMI in this young group is very important in providing accurate diagnosis and prompt management of the disease. The discovery of miRNAs in the AMI pathogenesis in this young population could lead to their potential usage as novel biomarkers for detection of early cardiac injury, providing prognosis and predicting development of complications following AMI as well as for therapeutic intervention. Therefore, this warrants further studies in this area.

1.2 RESEARCH QUESTION

How miRNAs involve in the pathogenesis of AMI in young AMI group in our population and how do they affect the expression and translation of genes related to the pathophysiology of AMI in this young population?

1.3 GENERAL HYPOTHESIS

There is an involvement of miRNAs in AMI event of young adults in our population.

1.4 SPECIFIC HYPOTHESES

i. There are specific miRNA profiles that are present in AMI patients in our population.

ii. There are different pathways that involve in the pathogenesis of AMI in Young AMI group based on the significantly dysregulated miRNAs.

iii. The miRNAs are differently dysregulated in Young AMI and Mature AMI.

iv. The mRNA expressions of the dysregulated miRNAs in AMI event are differently dysregulated between Young AMI and Mature AMI.

1.5 GENERAL OBJECTIVE

The general objective of this study was to investigate the involvement of miRNAs in AMI of young adults in Kuantan, Pahang.

1.6 SPECIFIC OBJECTIVES

v. To profile miRNAs in Young AMI and Mature AMI patients.

vi. To identify the pathway involves in pathogenesis of AMI in Young AMI group based on the dysregulated miRNAs.

vii. To compare the miRNAs that are dysregulated between Young AMI, Mature AMI, and Control group.

viii. To measure the mRNA expressions of dysregulated miRNAs in AMI event between Young AMI and Mature AMI.