PROFILE OF FINAL REPORT

Project ID/Title: FRGS/1/2019/SKK08/UIAM/02/3 / THE IDENTIFICATION OF microRNAs PATHOGENIC PATHWAY IN ACUTE MYOCARDIAL INFARCTION OF YOUNG ADULTS

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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 $\leq$ 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 $\leq$ 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.

Keywords:
microRNA (miRNA); Acute Myocardial Infarction (AMI); Young AMI; AMI Pathogenesis

Introduction:

Acute myocardial infarction (AMI) is the leading cause of death worldwide. The incidence of AMI is increasing in recent years, and it is associated with younger age where the prevalence in young population less than 40 to 45 years old ranged between 2 and 10 percent around the world (Andersson et al., 2018; Shih et al., 2020). Current data from the Department of Statistics Malaysia, showed that the prevalence of AMI in adults aged 41-59 years old is 20% and in adults aged more than 60 years old is 18% (Department of Statistic Malaysia, 2021).

One of the major factors for developing AMI in this young population is genetic predisposition. A family history of coronary heart disease is considered as one of the most relevant risk factors for the early onset of AMI. Therefore, a deeper molecular understanding on the pathological processes of acute myocardial ischaemic damage and infarction are crucial for both basic cardiovascular and clinical research. Since the study of ischaemic heart disease in young population is important in the era of preventive cardiology, this knowledge is also crucial in developing the framework in primary & secondary prevention.

Specific miRNAs are postulated to be involved in various stages of AMI pathogenesis in cell culture & animal studies (Ge et al., 2019; Guo et al., 2020; Hao et al., 2020; Shi et al., 2020). However, their complex regulatory mechanisms have not been completely understood (Schulte et al., 2017). 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 young adults. Understanding the pathogenesis of AMI in this young group is very important in providing accurate diagnosis and prompt management of the disease. Therefore, this warrants further studies in this area.

miRNAs are short, noncoding RNAs that regulate gene expression post-transcriptionally by base pairing to partially complementary sequences in target messenger RNA (mRNA) (Wang et al 2015). In AMI, miRNAs possibly affect the atherogenesis, a precursor for AMI by affecting the genes that regulate endothelial stability & atherosclerotic plaque destabilization. miRNAs may also affect the genes involved in the pathogenic pathway of AMI including apoptosis, necrosis & autophagy. However, information on these theoretical roles of miRNA

is scarce. Therefore, it is important to dissect further miRNAs involvement in pathogenesis of AMI in this young population.

The discovery of miRNA in the AMI pathogenesis in this young population could lead to its potential usage as a novel biomarker for detection of early cardiac injury, providing prognosis & predicting development of complication following AMI as well as for therapeutic intervention.

Background:

Coronary artery disease (CAD) is the leading cause of death worldwide. Acute myocardial infarction (AMI) is the lethal manifestation of CAD and can present as sudden death. According to the World Health Organization (WHO), in 2019, 17.5 million people died from cardiovascular disease (WHO, 2021). Of these deaths, 85% were due to AMI and stroke.

In Malaysia, AMI is also the leading cause of death and accounted for 17% of 109,155 medically certified deaths in 2020, raising by 2% from 2019 (Department of Statistics Malaysia, 2021). The National Cardiovascular Disease Database (NCVD) showed that Malaysians are having acute coronary syndrome (ACS) at much younger age (mean age between 55.9 to 59.5 years) compared to most developed countries (mean age 63.4 to 68 years) (Lu et al., 2014).

Framingham Heart Study defined young as age 30 to 44 years old (Andersson et al., 2021. In most of other published studies, the definition of young patients includes individuals less than 45 years old (Abed et al., 2018; Mansour et al., 2020; Shih et al., 2020; Younis et al., 2020). Therefore, in this study, young AMI patient was taken as 45 years old or younger. According the Malaysian 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). Although these patients only account for minor proportion of all patients with AMI, this population is of particular interest due to the long-life expectancy. Besides, the consequences of AMI can be devastating particularly at "young" age due to greater potential impact on patient's psychology, ability to work & the socioeconomic burden. As young AMI patient may be the main income producer of the family, the aftermath of AMI can also affect multiple dependents.

Obtaining novel insight into the pathophysiology of AMI could give better understanding of this disease and potentially aid the discovery of new biomarkers for early detection and therapeutic intervention, as well as for determining the prognosis following an AMI.

Circulating biomarkers of myocardial damage, especially cardiac specific troponin is the current gold standard biomarker for early diagnosis of AMI (Rajadurai et al., 2019; Thygesen et al., 2019). However, a variety of medical conditions including heart and renal failure, severe sepsis and atrial fibrillation can enhance the circulating troponins in the absence of AMI leading to false positive result (Chaulin 2022; Park et al., 2017). In AMI, troponin levels usually increase as early as 3.5 hours after the onset of chest pain (Park et al., 2017). However, due to the relative delay in the release of troponin, the search for more specific and sensitive biomarkers are crucial and needed to improve early AMI diagnosis and further reduce the AMI mortality, particularly in patients with atypical symptoms and uninterpretable echocardiogram (ECG).

MiRNAs have been shown to be involved in biological processes in mammals & humans including disease development such as AMI. miRNAs are highly conserved, endogenous, small (19-25 nucleotides long) noncoding RNAs that negatively regulate gene expression post-transcriptionally, by recognizing complementary mRNAs and prohibiting their translation into functional protein and thus affecting a variety of cell processes (Schulte et al., 2017). miRNAs are expressed in most tissues where they regulate multiple pathophysiological processes. Altered miRNA expression patterns have been associated with various cardiac pathologies (Schulte et al., 2017). However, functional specificity for miRNAs in the pathogenesis of AMI is still lacking. Therefore, identifying more specific miRNAs involved is crucial in understanding the disease process.

2. MicroRNAs & AMI

AMI is defined pathologically as myocardial cell death due to prolonged ischaemia and it is the most severe manifestation of coronary artery disease (CAD). The development of CAD can be chronic due to endothelial erosion and plaque buildup, which leads to the narrowing of the coronary artery. This results in transient cardiomyocyte ischaemia and usually presents clinically as angina or chest pain. However, a sudden atherosclerotic plaque rupture and thrombus formation usually leads to AMI, which is more serious and can be fatal. Once the

oxygen supply is occluded, the onset of AMI is initiated after the first 20 minutes and within a few hours, complete myocardial necrosis happens (Sun et al., 2017). Prolonged ischaemia could lead to the loss of heart contractility due to poor proliferation capability of myocardial cells. Therefore, a timely revascularization of the occluded coronary artery is the key for AMI therapy.

AMI causes dramatic changes in the structure and composition of the heart tissues. After initial necrosis of the ischaemic zone, the remodeling process and cellular replacement is set in motion, triggering a dynamic process characterized by changes in gene expression and cellular composition (Sun et al., 2017; Tanase et al., 2021). Different studies in animal models have shown that there are several cardiac miRNAs that were dysregulated following permanent coronary artery occlusion (Li et al., 2021; Zhang et al., 2021; Huang et al., 2021; Ma et al., 2021). The only studies on human were done on cardiac tissues obtained from postmortem (Bostjancic et al., 2009; Bostjancic et al., 2010). Other studies look at the circulating miRNAs that were released following an AMI (Xue et al., 2019; Li et al., 2019; Wang et al., 2020; Zhang et al., 2020). These studies provided a long list of modulated miRNAs but in most cases did not address the specific role of selected miRNAs in AMI.

The complex regulatory mechanisms involved in miRNA and gene expression have not yet been completely understood and elucidated. Their discovery could lead to its potential usage as new biomarker for early detection of AMI as well as for identifying prognosis following AMI & for target of molecular intervention in management of AMI in the future.

Objectives:

General Objective

To investigate the involvement of miRNAs in AMI of young adults in Kuantan, Pahang.

Specific Objectives

1. To profile miRNAs in Young AMI and Mature AMI patients.
2. To identify the pathway involves in pathogenesis of AMI in Young AMI group based on the dysregulated miRNAs.
3. To compare the miRNAs that are dysregulated between Young AMI, Mature AMI, and Control group.

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

Methodology:

This study is comparative cross-sectional study between control and AMI.

1. Ethical Approval

The human ethical approval was sought before the commencement of the study.

2. Sample size

Openepi software was used for the sample size calculation. For this study, a power of 80% was chosen for it to have a relevant effect with confidence interval of 95%. For profiling of miRNA, the proposal refers to previous genome wide array for profiling of miRNA in various chronic diseases that reported the use of sample size around 20s between subjects and controls. Objective 1 of the study was used for the sample size calculation. A study by Huang et al. (2014) was selected, where the mean and standard deviation (SD) of miR-320, which was significantly dysregulated between control and AMI were used. After adding 20% for any possible drop out, the final sample size was 20 for each group, with one-to-one ratio.

3. Subjects and Sample Collection

There were 3 groups in this study where Group 1 was Control (age 18-45 years old), Group 2 was Young AMI (age ≤ 45 years old) and Group 3 was Mature AMI (age > 45 years old). The criteria for healthy controls were Malaysian aged 18 to 45 years who were healthy with no known chronic illnesses, alcohol consumer or on any medication. The inclusion criteria for AMI groups were Malaysian with first episode of clinically confirmed STEMI, age ≤ 45 years for Young AMI group and age ≥ 46 for the Mature AMI group. Exclusion criteria for AMI groups were any prior thrombolytic therapy or percutaneous intervention and other known chronic diseases, alcohol consumer as well as those on any medication. STEMI is defined by local guidelines as elevation of ST segment ≥ 1 mm in two contiguous electrocardiographic (ECG) leads or the presence of new left bundle branch block (LBBB) with positive cardiac enzymes (Rajadurai et al., 2019)

Healthy control who fulfilled the study criteria was recruited during health screening at Klinik Kesihatan in Kuantan or Clinical Trial Unit (CTU) in IIUM. Once informed consent was taken, an appointment date at CTU was given where blood sample was taken during the appointment.

For AMI patients, the recruitment was done at the Emergency Department (ED) of HTAA and ED of SASMEC@IIUM within 12 hours of the onset of AMI symptoms. Once informed consent was given by the patient, blood sample was taken.

Basically, AMI patients and normal controls who fulfilled the study criteria and consented were enrolled into the study. 2 mls of venous blood was taken by the experienced phlebotomist. The study was conducted following the Declaration of Helsinki (World Medical Association, 2013) and guidelines from Ethical Committee of Kulliyyah of Medicine, IIUM (IIUM/305/20/4/1/7) and Medical Research and Ethical Committee (MREC), Kementerian Kesihatan Malaysia (NMRR-16-2572-32869 (IIR)).

4. miRNA preparation

Plasma will be separated by centrifugation and immediately stored at -80 degree Celsius. On preparing total RNA and miRNA, the plasma will be thawed and subjected to several steps of spin column (Qiagen miRNAeasy®) procedures to get the purified miRNA and total RNA.

5. miRNA profiling

Profiling was done using plasma of Young AMI & Mature AMI subjects as well as normal controls through small RNA (sRNA) sequencing. The library construction and sRNA sequencing were performed using BGISEQ500 SE50 (BGI, Shenzen, Guangdong, China). Small RNA libraries were constructed using BGI protocol. Small RNAs were enriched and purified and the 3' end adapter was ligated. Then Unique molecular identifier (UMI) labelled Primer was added followed by the digestion of the unligated adaptors and 5' end adaptor ligation. Next the cDNA was synthesized with UMI labelled primer followed by fragment selection. The ligation product was then amplified and subjected to the single-strand circularization process, deriving single-strand circular DNA library. Following the library quality control (QC), the single strand circular DNA library was amplified using PCR as per manufacturer's protocol to produce DNA NanoBalls (DNBs). Next the DNBs were loaded onto the sequencing chip and finally sequencing was done using BGISEQ500 SE50 platform at BGI (Shenzen, Guangdong, China). After filtering the raw data, the remaining clean data

was stored in FASTQ format. Bowtie2 was used to map the clean data to the reference genome and other sRNA databases including miRbase, primabank, snoRNA, Rfam and also miRDeep2. RNAhybrid, TargetScan and miRanda were used to find the target gene of miRNAs. DEGseq method was used to analyse the differentially expressed SRNAs (DESs). DEGseq method was used to analyse the differentially expressed miRNAs between the 3 groups. P-value was adjusted with the q-value where any q-value < 0.05 and [log2(fold change)] > 1 was put as the threshold for the significantly differential expression by default.

Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) Enrichment Analysis

For the gene ontology (GO) enrichment analysis, all genes were mapped to the GO-terms in the database (http://www.geneontology.org/) according to the principle of GO classification (Ye et al., 2018). All the information were annotated and classified according to the biological process, molecular function, and cellular components. Kyoto Encyclopaedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/), a major public pathway-related database, was used to perform the pathway enrichment analysis where it identified significantly enriched metabolic pathways or signal transduction pathways in the target genes when compared to the whole genome background (Kanehisa et al., 2008). These pathways were classified into metabolism, genetic information processing, environmental information processing, cellular processes, organismal systems, human diseases, and drug development where each category was further divided into sub-classes.

6. Quantitative real-time PCR (qRT-PCR)

The upregulated and downregulated miRNA were tested in individuals and were validated using qRT-PCR using the standard protocol. The validation steps include selection of miRNAs based on small RNA profiling, conversion of RNA to cDNA, qRT-PCR and analysis of data using GeneGlobe software (https://geneglobe.qiagen.com/us/analyze) (Qiagen, 2020).

7. The assessment of mRNA and its link with the pathophysiology of AMI.

The positive upregulated miRNA was referred to mRNA databases. The link between miRNA and its mRNA were assessed further by assessment of the absolute gene mRNA expression. Basically, in this procedure mRNA was converted to cDNA and subjected to fluorescent-based RT-PCR. Standard curve was built with known standard mRNA of the gene of interest.

Absolute copies of mRNA were then determined. All procedures were in accordance with the MIQE guidelines for performing qRT-PCR.

Findings:

1. miRNA EXPRESSION PROFILE DATA

a) miRNA Expression Profile of Healthy Controls Versus AMI (Young AMI and Mature AMI) Patients.

The distribution of differentially expressed miRNAs revealed a total of 1599 miRNAs that were differentially expressed in AMI patients compared to Healthy controls, of which 1288 miRNAs were up-regulated, and 311 miRNAs were down-regulated (Figure 1).

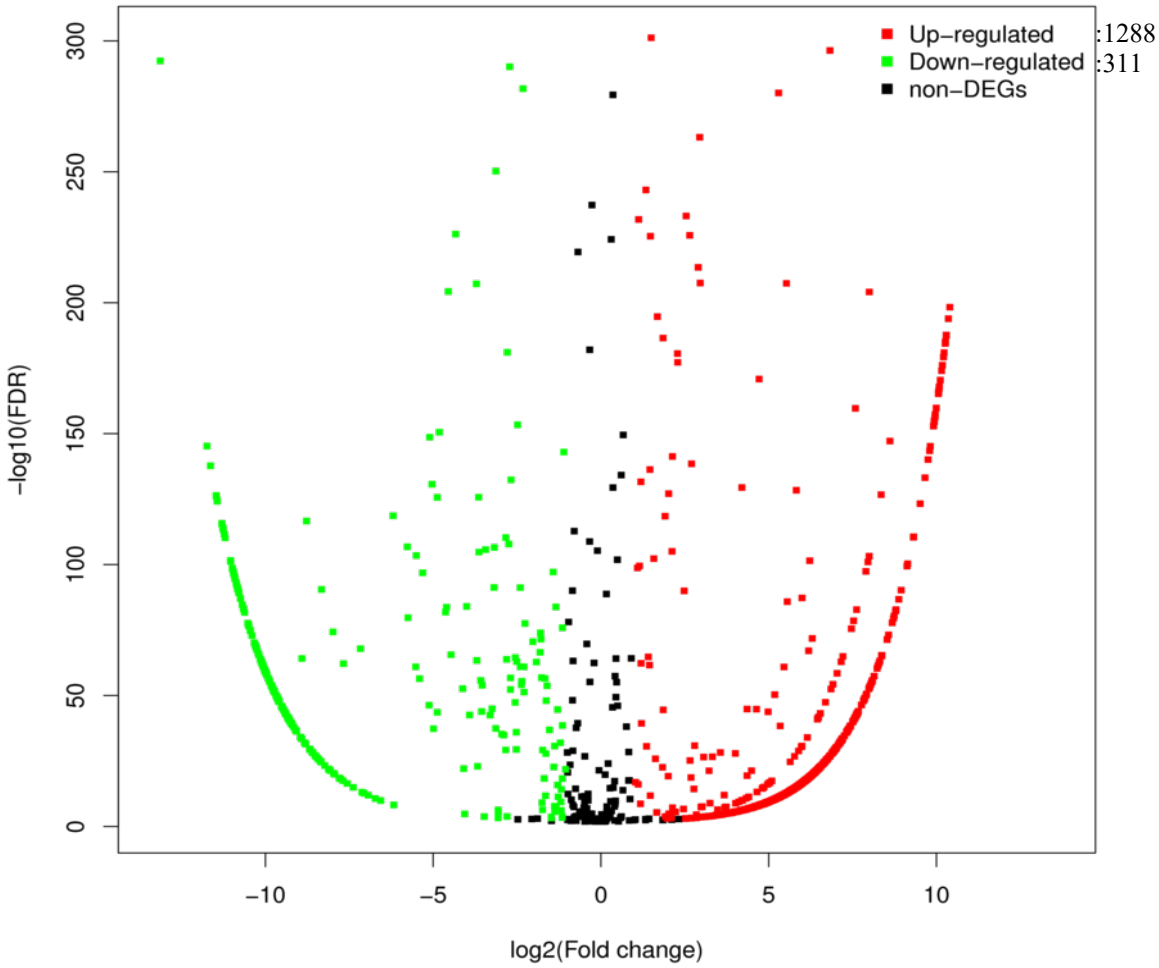

Figure 1: Volcano Plot of Differential miRNA Expression in Controls and AMI (Young AMI and Mature AMI) Patients.

Note: X-axis represents $\log_2$ fold change; Y-axis represents $-\log_{10}$ (corrected q-value) for each probe. Green points indicating down-regulation (log2(Fold change) $\leq$ -1 and FDR $\leq$ 0.001) and red points indicating up-regulation (log2(Fold change) $\geq$ 1 and FDR $\leq$ 0.001).

b) miRNA Expression Profile of Healthy Controls Versus Young AMI Patients.

The distribution of differentially expressed miRNAs revealed a total of 738 miRNAs that were differentially expressed in Young AMI patients compared to Healthy controls, of which 405 miRNAs were up-regulated, and 333 miRNAs were down-regulated (Figure 2).

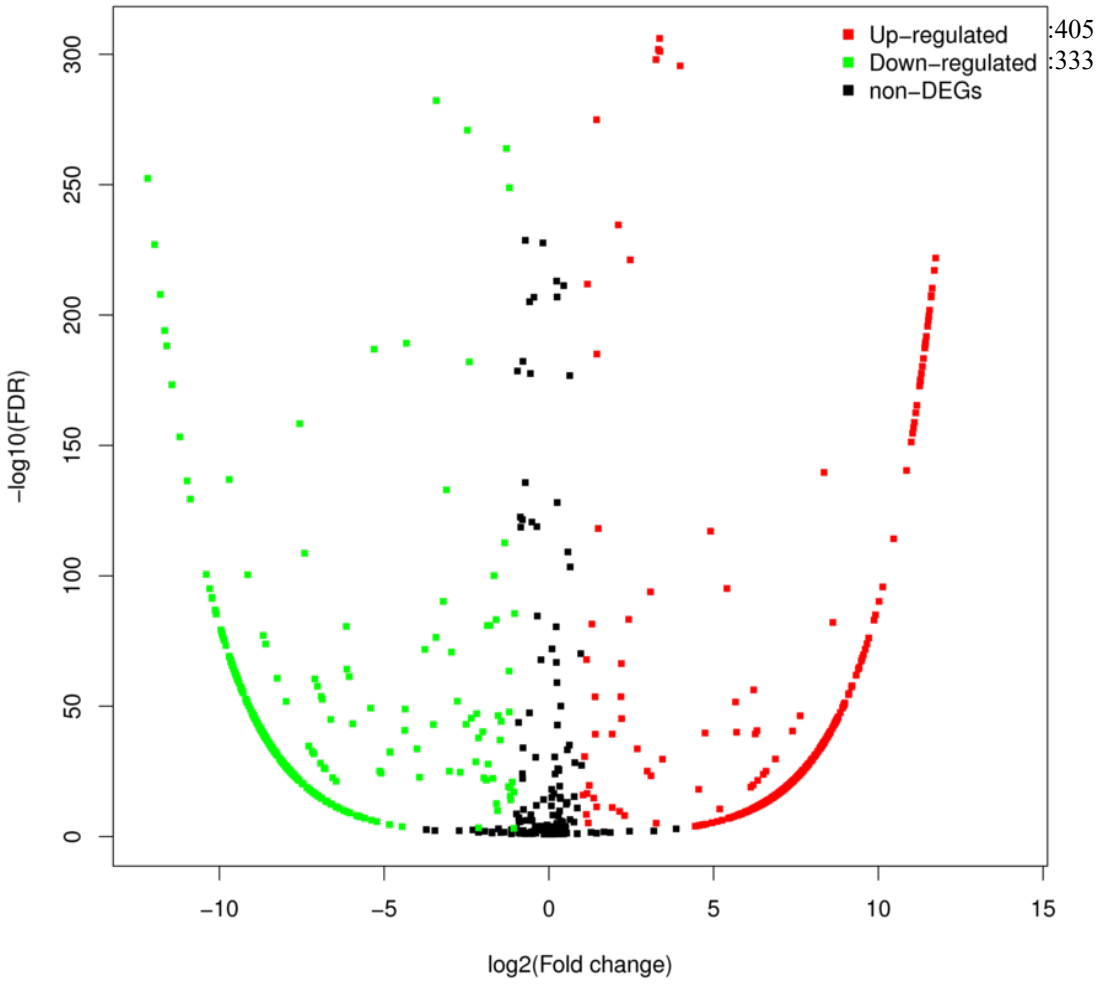

Figure 2: Volcano Plot of Differential miRNA Expression in Controls and Young AMI Patients.

Note: X-axis represents $\log_2$ fold change; Y-axis represents $-\log_{10}$ (corrected *q*-value) for each probe. Green points indicating down-regulation (log2(Fold change) ≤ -1 and FDR ≤ 0.001) and red points indicating up-regulation (log2(Fold change) ≥ 1 and FDR ≤ 0.001).

c) miRNA Expression Profile of Young AMI Versus Mature AMI Patients.

The distribution of differentially expressed miRNAs revealed a total of 1497 miRNAs that were differentially expressed in Young AMI patients compared to Mature AMI, of which 1090 miRNAs were up-regulated, and 407 miRNAs were down-regulated (Figure 3).

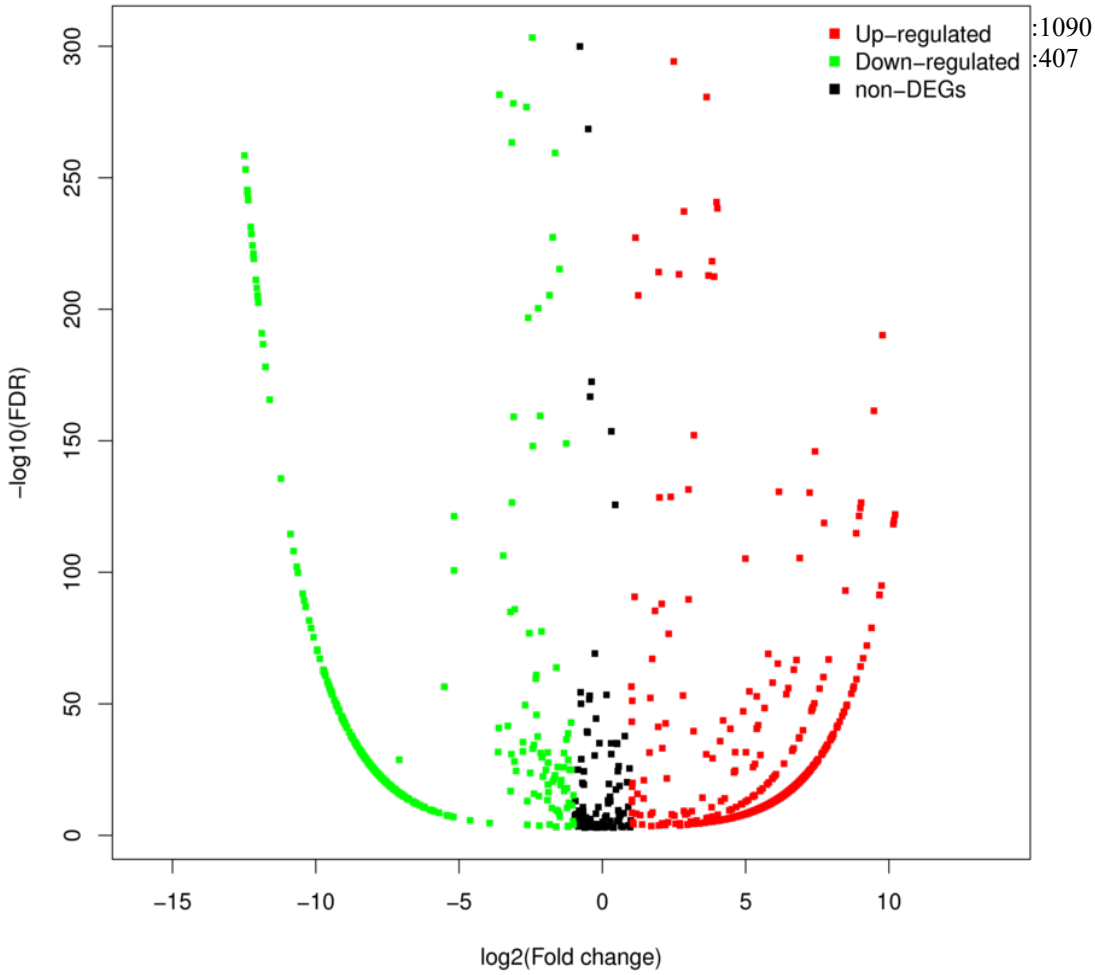

Figure 3: Volcano Plot of Differential miRNA Expression in Young AMI and Mature AMI Patients.

Note: X-axis represents $\log_2$ fold change; Y-axis represents $-\log_{10}$ (corrected *q*-value) for each probe. Green points indicating down-regulation (log2(Fold change) $\leq$ -1 and FDR $\leq$ 0.001) and red points indicating up- regulation (log2(Fold change) $\geq$ 1 and FDR $\leq$ 0.001).

d) Twenty Most Differentially Expressed miRNAs.

Of these twenty most differentially expressed miRNAs, ten (10) were found to be novel miRNAs where four (4) miRNAs including miR-552, miR-219, miR-982 and miR-58 were up-regulated and six (6) miRNAs including miR-1064, miR-790, miR-1177, miR-201, miR-419 and miR-1103 were down-regulated (Table 1).

Table 1: Differentially Expressed miRNAs Between Young AMI and Mature AMI Patients in Small-RNA Sequencing.

miRNAs	Young AMI vs Mature AMI patients		
	Regulation	Fold change	*p*-value
miR-552#	Up	13.74	< 0.0001*
miR-4446-3p	Up	11.50	< 0.0001*
miR-432-5p	Up	10.57	< 0.0001*
miR-548j-5p	Up	10.21	7.65E-121*
miR-219#	Up	10.18	1.39E-118*
miR-982#	Up	10.16	3.86E-117*
miR-181a-2-3p	Up	10.09	< 0.0001*
miR-654-5p	Up	9.78	4.62E-189*
miR-58#	Up	9.74	9.55E-94*
miR-548k	Up	9.67	2.90E-90*
miR-16-5p	Down	-15.91	< 0.0001*
miR-1064#	Down	-12.49	1.69E-257*
miR-431-5p	Down	-12.45	4.13E-252*
miR-790#	Down	-12.39	2.42E-244*
miR-1177#	Down	-12.38	2.52E-243*
miR-201#	Down	-12.38	3.1E-243*
miR-105-5p	Down	-12.36	3.48E-241*
miR-518	Down	-12.35	1.81E-240*
miR-419#	Down	-12.27	2.95E-230*
miR-1103#	Down	-12.25	1.20E-227*

Note. Unpaired T-test; *Significant difference at 95 % confidence interval, with fold change ≥ 1 or fold change ≤ 1. # Signifies Novel miRNAs.

2. PATHWAYS INVOLVE IN PATHOGENESIS OF AMI IN YOUNG AMI GROUP BASED ON DYSREGULATED miRNAs

   For the 1497 miRNAs that were differentially expressed, 34,195 target genes were predicted by GO analysis. The functional analysis revealed that 11,199 GO terms found to be involved in biological processes, 12,012 in cellular components, and 10,984 in molecular functions (Figure 4), were significantly enriched ($p < 0.05$). The most common GO categories were cellular process, single-organism process, metabolic process, biological regulation, response to stimulus, regulation of biological process, multicellular organismal process, cell, cell part, organelle, membrane, organelle part, membrane part, binding, and catalytic activity (Figure 4).

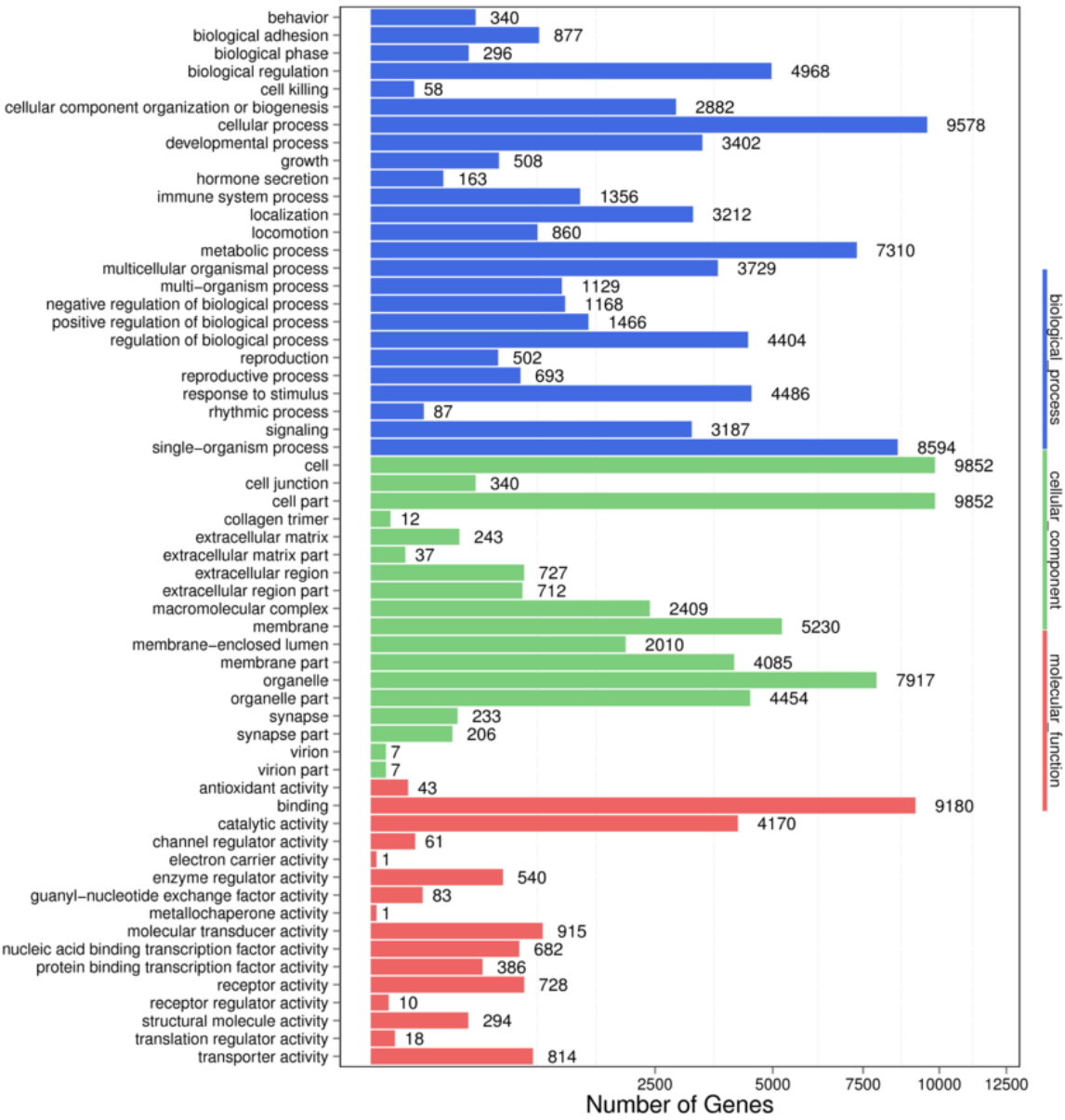

Figure 4: GO Analysis of Differentially Expressed miRNAs That Covers Three Domains: Biological Process, Cellular Components, and Molecular Function.

Note: X-axis represents Number of Genes (miRNAs). Y-axis on the left represents GO terms of biological process, cellular component, and molecular function. The blue row indicates the biological process, the green row indicates cellular component, and the red row indicates molecular function.

Comparison of biological pathway between Young AMI and Mature AMI identified 346 categories that were enriched. The top 20 pathways according to p-value are displayed in Figure 5. Of these 20 pathways, ascorbate and aldarate metabolism, glycosaminoglycans – heparan sulfate/heparin metabolism, and collecting duct acid secretion were the most significant pathways (Figure 5).

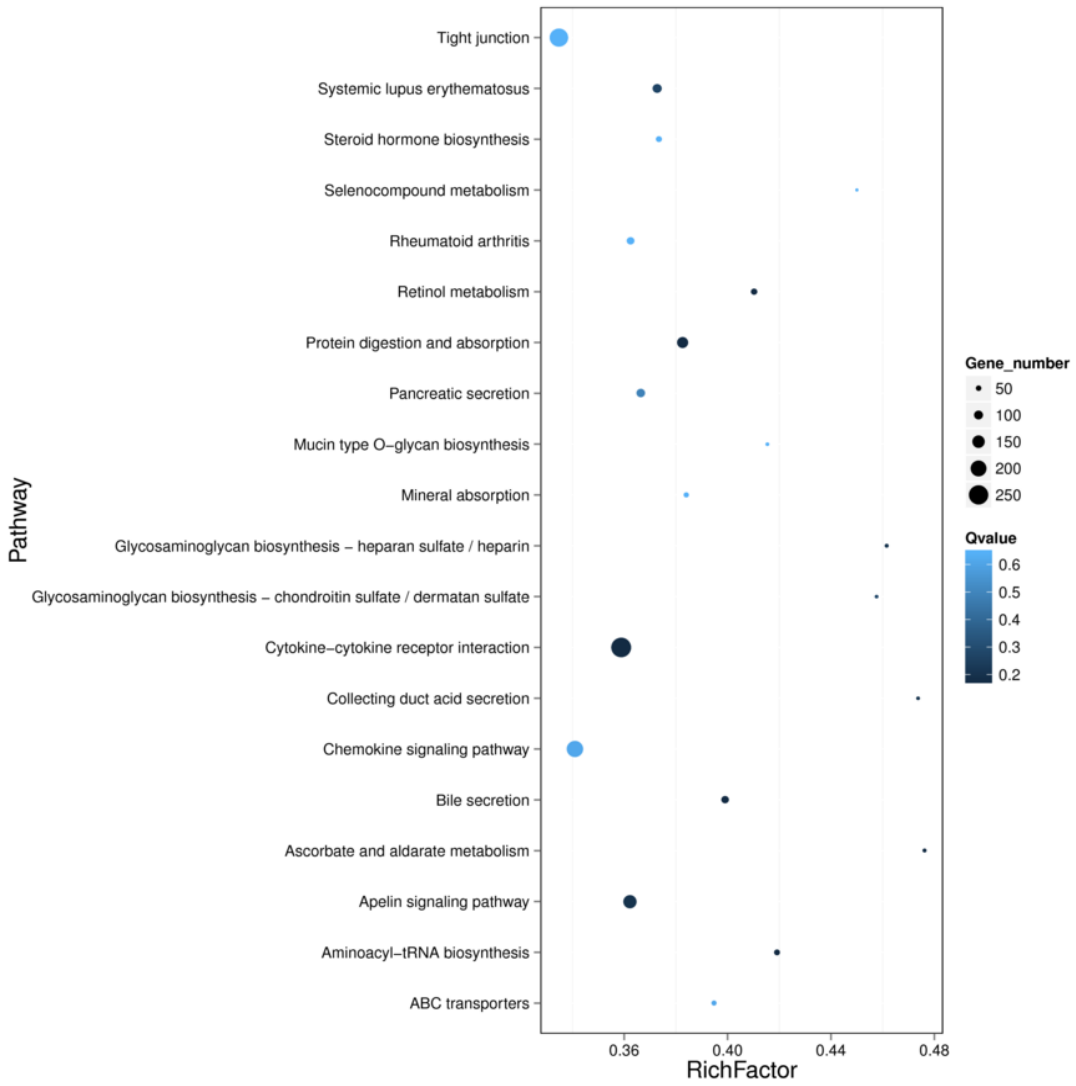

Figure 5: Scatter Plot of Enriched KEGG Pathway Analysis of Differentially Expressed miRNAs Between Young AMI and Mature AMI Patients.

3. VALIDATION OF DYSREGULATED miRNAs EXPRESSION IN HEALTHY CONTROLS, YOUNG AMI AND MATURE AMI PATIENTS

a) miRNAs Expression Between Heathy Controls and Young AMI Patients.

The GeneGlobe analysis revealed that there was a significant difference of miR- 423-5p (2.08-fold up-regulation, p = 0.048) expression between Healthy Controls and Young AMI patients. There were no significant differences of the other miRNAs (Table 2).

Table 2: Validation of miRNAs That Were Dysregulated Between Healthy Controls and Young AMI Patients

miRNA	Healthy Control ΔC_T	YAMI ΔC_T	Fold change	Regulation	*p*-value
miR-423-5p	12.82	11.76	2.08	Up	0.048*
miR-363-3p	12.69	12.54	1.11	Up	0.190
miR-431-5p	-1.68	3.61	-39.14	Down	0.120
miR-105-5p	-18.98	-18.77	-1.16	Down	0.0004
miR-378a-5p	7.30	3.78	11.45	Up	0.364
miR-16-5p	5.42	4.93	1.40	Up	0.080

Note. Paired T-test. The relative miRNA levels were normalized to *UniSp6*; *Upregulation ≥ 2 folds or downregulation ≤ 2 folds, with $p < 0.05$ are taken as significantly different.

b) miRNAs Expression Between Young AMI and Mature AMI Patients.

The GeneGlobe analysis revealed that there were significant differences of miR-431-5p (33.9-fold down-regulation, p = 0.034) and miR-378a-5p (-34.61-fold down- regulation, p = 0.040) expressions between Young and Mature AMI. There was no significant difference of the other miRNAs.

Table 3: Validation of miRNAs That Were Dysregulated Between Young AMI and Mature AMI Patients

miRNA	YAMI ΔC_T	MAMI ΔC_T	Fold change	Regulation	*p*-value
miR-423-5p	12.59	11.76	1.77	Upregulated	0.228
miR-363-3p	8.90	12.54	-12.46	Downregulated	0.102
miR-431-5p	-1.146	3.62	-33.90	Downregulated	0.034*
miR-105-5p	-18.59	-18.77	1.13	Upregulated	> 0.05
miR-378a-5p	-1.32	3.79	-34.61	Downregulated	0.040*
miR-16-5p	4.91	4.93	-1.01	Downregulated	0.075

Note. The relative miRNA levels were normalized to *UniSp6*; *Upregulation ≥ 2 folds or downregulation ≤ 2 folds, with $p < 0.05$ are taken as significantly different.

4. mRNA EXPRESSION OF THE DYSREGULATED miRNAs IN YOUNG AMI AND MATURE AMI PATIENTS

   The genes selected for mRNA expression assay using qRT-PCR based on the most dysregulated miRNAs in profiling result, were glutamate metabotropic receptor 4 (GRM4), sodium voltage-gated channel type 4 alpha (SCN4A) and membrane-associated ring-CH finger protein 6 (MARCH6). GRM4, SCN4A and MARCH6 were inadequately expressed in Young and Mature AMI patients.

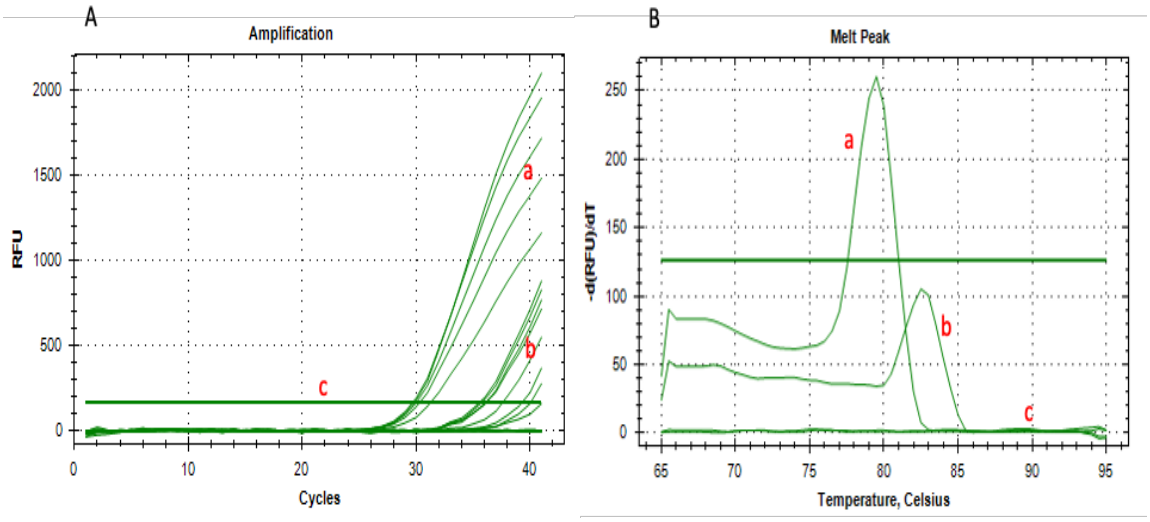

Figure 6: Amplification Curve (A) and Melt Curve (B) of the Target and Housekeeping Genes at Various Temperatures in Gradient Analysis. Late amplification identifies for *MARCH6* (a) and *GAPDH* (b). No amplification for the rest of target and reference genes (c).

Conclusion:

The present study showed that:

1. There are specific miRNA profiles that are present in AMI patients in our population in Kuantan, Pahang. There were 1599 miRNAs that were differentially expressed in AMI (Young and Mature AMI) patients where majority miRNAs (1288) were upregulated. There were ten novel miRNAs implicated in Young AMI against Mature AMI patients based on the top ten dysregulated miRNAs in the profiling analysis where their functions in AMI pathogenesis are still unknown.
2. Ascorbate and aldarate metabolism, glycosaminoglycans biosynthesis – heparan sulfate/heparin, and collecting duct acid secretions pathways are involved in the pathogenesis of Young AMI. However, the exact mechanisms of how these pathways involve in the pathogenesis of AMI in young population require further clarification.

3. Validation on miRNAs expressions showed miR-423-5p was over-expressed in Young AMI patients while miR-431-5p and miR-378a-5p were under-expressed in Mature AMI patients. miR-423-5p is the potential biomarker for early cardiac injury in young population whereas miR-431-5p and miR-378a-5p are the potential target treatment for AMI.
4. GRM4, SCN4A and MARCH6 were inadequately expressed in Young and Mature AMI patients.

There are roles of miRNAs in AMI event of young adults in Malaysian population. However, further functional cell lines and animal studies are needed to determine the roles of these miRNAs in AMI pathogenesis and how these miRNAs influence the ascorbate and aldarate metabolism, glycosaminoglycans biosynthesis – heparan sulfate/heparin, and collecting duct acid secretion pathways in AMI event in these young population. Moreover, larger multi-centre studies are required to confirm the significant miRNAs as biomarkers of AMI in young patients.

Output:

a) Human Capital Development

Details of Student

Student Full Name: Nurul Ashikin Binti Muhammad Musa
IC/Passport No: 751223-14-5918
Student ID: G1710878
Citizenship: Malaysian
Year of Graduation: 2022
Thesis Title: MicroRNA (miRNA) Profile in Acute Myocardial Infarction (AMI) of Young Adults in Kuantan, Pahang

Student Full Name: Iffah Irdina Binti Mohd Zamri
IC/Passport No: 970528-06-5580
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Year of Graduation: Not graduated yet (currently in the process of completing thesis writing)

Thesis Title: A Pilot Study on Association of LPA Gene Copy Number Variation (CNV) and Apolipoprotein E (APOE) Gene Polymorphism in Acute Myocardial Infarction (AMI) of Young Adults

b) Publication:

Indexed Journal:

1. Nurul Ashikin Muhammad Musa, Nor Zamzila Abdullah, Norlelawati A. Talib, Azarisman Shah Mohd Shah, Aszrin Abdullah and Aida Nur Sharini Mohd Shah, **microRNAs Expression Profile In Young Patients with Acute Myocardial Infarction**
   IIUM Medical Journal Malaysia (IMJM), Volume 21(4). October 2022. http://doi.org/10.31436/imjm.v21i4.2108

2. Iffah Irdina Mohd Zamri, Nor Zamzila Abdullah, Norlelawati A.Talib, Aszrin Abdullah, Nurul Ashikin Muhammad Musa, **LPA Gene Copy Number Variation and APO E Gene Polymorphism in Young Acute Myocardial Infarction**
   Submitted to IIUM Medical Journal Malaysia (IMJM) and status still pending.

c) Intellectual Property: No

d) Additional Outputs

Conference:

1. Nurul Ashikin Muhammad Musa, Nor Zamzila Abdullah, Norlelawati A. Talib, Aszrin Abdullah, Azarisman Shah Mohd Shah
   The Role of miRNA in Acute Myocardial Infarction of Young Adults. Gold Award. Poster presented at Kuantan Research Day 2020 (KRD2020), International Islamic University Malaysia. 1st October – 5th January 2021.
   Presented at Kuantan Research Day 2020 (KRD2020), International Islamic University Malaysia on 1st October – 5th January 2021.

2. Nurul Ashikin Muhammad Musa, Nor Zamzila Abdullah, Norlelawati A. Talib, Aszrin Abdullah, Aida Nur Sharini Mohd Shah, Azarisman Shah Mohd Shah.
   The miRNAs Expression Profile in Acute Myocardial Infarction of Young Adults. Second Place Non-Clinical Poster.

Poster presented at the Virtual Medical Research Symposium 2021 (MRS2021) on 14th December 2021.

3. Iffah Irdina Mohd Zamri, Nor Zamzila Abdullah, Norlelawati A. Talib, Nurul Ashikin Muhammad Musa, Aszrin Abdullah.
   The Association of LPA Gene Copy Number Variation and Apolipoprotein E (Apo E) Gene Polymorphism in Young Acute Myocardial Infarction. First Place Non-Clinical Poster.
   Poster presented at the International Virtual Medical Research Symposium 2022 (MRS2022) on 15th December 2021.

Future plan of the research:

1. Verify if there are any differences in the expression of miR-423-5p, 431-5p and miR-378a-5p among Young and Mature AMI patients between different races in Malaysia before they can be considered as useful biomarkers for early cardiac injury in AMI.
2. Do functional studies in cell lines and animal models on the roles of these novel miRNAs in AMI pathogenesis and how they influence the ascorbate and aldarate metabolism, glycosaminoglycan biosynthesis -heparan sulfate/heparin, and collecting duct acid secretion pathways in AMI event among young population.
3. Do large multi-center studies to confirm the significance of these miRNAs as biomarkers of AMI in young patients.

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