Study Protocol

Circulating microRNAs as prognostic markers of ischemic heart disease

Rationale

Patients with stable ischemic heart disease (IHD) require regular follow-up visit to monitor the progression of disease and to understand the response to the treatment. This involves assessment of the level of blood based cardiac markers and echocardiography to measure cardiac function. While the echocardiogram is the gold standard method to fully assess cardiac function, it requires the patients to visit a speciality centre and is expensive, thereby restricting the frequent use of echocardiography to monitor cardiac disease status. However, recent studies demonstrate that cardiac disease can progress rapidly, especially in patients with underlying disorders such as diabetes or hypertension, who require constant monitoring and repeated echocardiogram measurements. Therefore, a biomarker that can be easily tested to monitor the progression of the disease and response to the treatment is likely to be highly beneficial.

MicroRNAs (miRs) are endogenous, short (20-22 nucleotides), tissue-specific, non-coding RNA molecules that regulate gene expression at the posttranscriptional level. miRs negatively regulate gene expression by promoting degradation or repressing the translation of target mRNA into protein, and play an important role in a wide range of physiological and pathological processes.[1-3] While the majority of miRs are found intracellularly, a significant number of stable miRs have been observed in various body fluids, exported via the miR transport system[4]. In support of this observation we and others have shown direct correlation of circulating cardiac specific miRs with the development of cardiovascular diseases using an animal model of diabetes. In addition, our recently concluded study showed a strong correlation with the level of circulating cardiac specific miRs and ejection fraction in patients undergoing cardiopulmonary bypass graft surgery (Fomison-Nurse et al, unpublished data, 2013). In addition, our on-going study with diabetic volunteers showed that the level of circulating miRs changes in response to the treatment for diabetes. These findings lead us to hypothesize that circulating miRs could become a reliable, quick, easy and inexpensive prognostic tool in patients with IHD. To date, there is no information regarding the prognostic impact of circulating miRs in cardiovascular disease, except one report showing direct correlation of miR-133a and miR-208 with increased mortality at 6 months in acute coronary syndrome patients.[5]

Study Aim

We aim to perform a proof-of-concept study on patients newly diagnosed with stable IHD and on medical treatment. We will measure the level of circulating miRs and cardiac function at the beginning and end of the study to demonstrate the sensitivity and specificity of the miRs as prognostic markers.

Recruitment

Recruitment will be conducted through Department of Cardiology at Dunedin Hospital under supervision of Prof M Williams. Potential participants (when first diagnosed with IHD) will be informed about the study during clinical visits and provided with an introductory letter and information sheet, with the opportunity to contact study investigators. Healthy volunteers (group e) will be recruited through public advertisement.

Study groups

Non-diabetic patients (n=64) newly diagnosed with ischemic heart disease with or without heart failure at Dunedin Hospital will be recruited after written consent.

Inclusion criteria

1. Non-diabetic newly diagnosed with ischemic heart disease.

2. Age - 20 – 80 years of both genders

Exclusion criteria

1. History of acute myocardial infarction

2. History of valvular disease.

3. Aged >80 years and < 20 years

4. Recent major surgery

5. Diagnosed with cancer

Sample size

Since this will be the first proof-of-concept study to demonstrate if miRs can be a prognostic marker, the sample size was estimated based on the published literature[6, 7] to detect the (a) difference between recruitment (time 0) and follow-up periods and (b) group difference between non-diabetic and diabetic with *alpha of 0.05, a power of 0.8 and a two-sided unpaired t-test using* GraphPad Statmate 2.

Protocol

1. ***Informed consent –*** Interested participants will be asked to sign consent.

2. ***Blood sampling procedures*** – Blood sampling will be performed by a trained nurse using standard aseptic technique. Six millilitres of total blood will be collected in the vacutainer containing EDTA (purple cap) and will be coded with a unique identification number. Blood samples will be collected at the time of the recruitment in to the study (time 0) and further follow-up samples will be collected at 6, 12, 24, 36, 48 and 60 months.

3. ***Separation of Plasma*** – Blood samples will be immediately transported to the Department of Physiology, where the samples will be centrifuged at 2000 rotations per minute for 10 minutes to separate the plasma. The plasma will be then collected in a new 1.5ml sterile eppendorff tube and stored in -80ºC freezer, which has accessibility only to the researchers. The residual pellet consisting of red blood cells and platelets will be disposed of securely as clinical waste.

4. ***Extraction of total RNA*** – Group of samples will be then used to extract total RNA using commercially available RNA extraction kit.

5. ***Reverse transcription of RNA to cDNA and real-time PCR amplification*** – The RNA will be then reverse transcribed using specific primers to each candidate miRs, followed by real-time PCR amplification.

6. ***Echocardiography assessment of cardiac function*** – In addition to routine echocardiography during the initial diagnosis of IHD, additional assessment of cardiac function will be conducted during the 6, 12, 24, 36, 48 and 60 months visit of the study participants. Echocardiography will be performed by a trained sonographer and the images will be reviewed offline under the supervision of Professor Michael Williams. The additional cardiac assessment is required to correlate the changes in the level of miRs to cardiac function.

7. ***Statistical analysis –*** Differences between groups will be tested with a multiple or two-way ANOVA, followed by a Bonferroni post-hoc test. Biostatistician will be consulted for further statistical analysis of the data..

References

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