Research Report

Introduction

An ageing and increasingly obese population heralds a global epidemic of diabetes, which poses a major risk to individual and public health. The World Health Organization estimated that 170 million people suffered from diabetes in 2000, and anticipates a doubling of global prevalence by 2030 as a consequence of population ageing and urbanization.[1] In New Zealand more than 208,000 people have been diagnosed to have diabetes to date and diabetes is considered an epidemic (Source – Diabetes NZ). These statistical details should alarm health care planners, since the prevalence of diabetic heart disease (DHD), which includes ischemic heart disease, heart failure and diabetic cardiomyopathy is responsible for more than 50% of deaths in diabetic patients.[2] In spite of many advances in the prevention of cardiovascular diseases, the outcomes in patients with DHD remain consistently poor, with sufferers effectively experiencing major vascular events at least 15 years before the non-diabetic population.[3] Moreover, once cardiovascular disease is clinically manifest, diabetes remains an independent risk factor for adverse outcomes, as noted in contemporary studies of myocardial infarction (MI) and chronic heart failure (CHF).[4, 5]

Recent studies have clearly shown that heart disease in diabetes develops at a much earlier stage before it is clinically diagnosed and therefore, timely management may halt the progression of the disease. Diastolic dysfunction in diabetes occurs as a consequence of molecular changes at gene level. These changes are attributed to the time-dependent change in cardiac gene expression [6-10]. Therefore, an attempt to diagnose the disease at molecular level can be a valuable strategy towards effective therapeutic management in patients with DHD. However, this requires the development of effective diagnostic tool for the early diagnosis of the disease. Currently, there are no specific and reliable tools to detect the early stages of the disease. The available clinically diagnostic tools such as echocardiography, coronary angiography and levels of circulating biomarkers can only diagnose established disease[11], suggesting the need for a novel non-ivasive tool to diagnose the diabetic heart disease at the early stage.

MicroRNAs (miRs) are endogenous, short (20-22 nucleotides) 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.[9, 12, 13] In addition to ubiquitous miRs, tissue specific miRs play a vital role in identifying the specific tissue pathology. This discovery of miR as regulators of molecular targets involved in pathogenesis of cardiovascular diseases has uncovered a new and exciting way for diagnosis of DHD. However, our knowledge on which miRs are altered in DHD is largely incomplete. Therefore, this project aimed at understanding the changes in the expression pattern of circulating cardiac specific miRs in people with diabetes without any known history of cardiovascular disease. Being a limited budget grant, we have not yet reached our final goal, although we have made clear advancement in our hypothesis, which has allowed us to apply for big project grants.

Methods

Ethical approval – This study was approved by the University of Otago Human Ethical Committee (ET16/13) and Human and Disabilities Ethics Committee (LRs/12/01).

Recruitment of volunteers – We recruited type-2 diabetic volunteers through direct mail and advertisement in the local newspaper. In addition, the PhD student involved in this project attended the diabetes clinic every week with Assoc Prof Patrick Manning to recruit the interested volunteers. To date we have collected samples from 88 non-diabetic and 45 type-2 diabetic volunteers with different duration of diabetes.

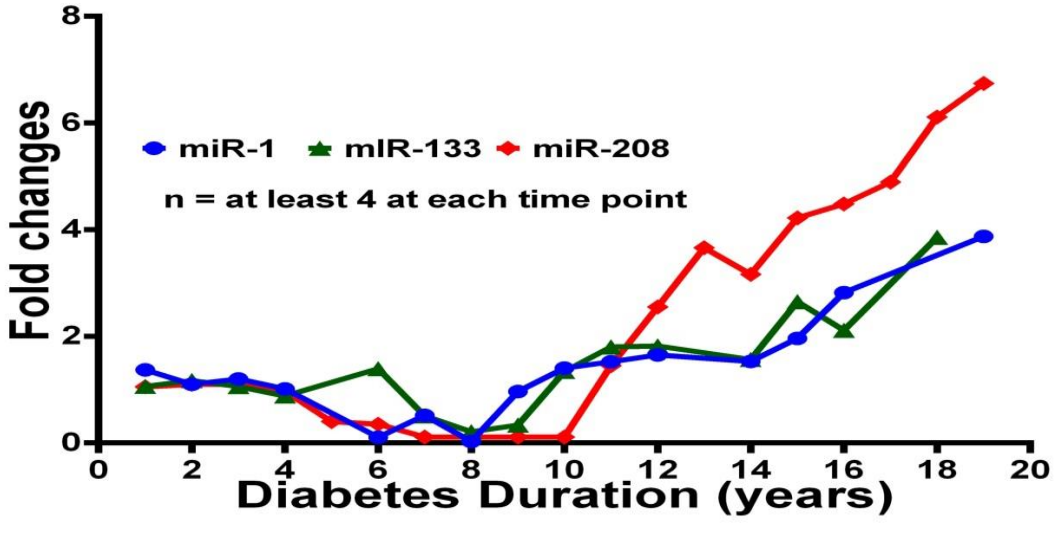
Blood collection – 2.5ml of blood samples were collected from the volunteers, plasma separated by centrifugation and stored at -80°C till further analysis.

Extraction of RNA and RT-PCR – Total RNA was extracted using the commercially available kit (Qiagen), the RNA was reverse transcribed to cDNA using target-specific stem loop structure and reverse transcription primers, and after reverse transcription, specific TaqMan hybridization probes was used to quantify the expression of miR-1, miR-208 and miR-133 (all from Applied Biosystems). The small RNA molecule U6 small nuclear (Rnu6-2, Applied Biosystems) was used as a control. Data was quantified by normalizing the amount of candidate miR to the amount of U6 miR using the 2−DDCT method. Each reaction was performed in triplicate.

Results and discussion

RT PCR analysis showed marked activation of cardiac specific miR-1, miR-208 and miR-133 in diabetics (**Figure 1**). These changes started at 12 years after the diagnosis of diabetes and progressively increased with the duration of the disease. Importantly, none of these volunteers had any history of known cardiovascular

Figure 1 – Cardiac specific circulating miRs over duration of diabetes (vs. non-diabetic)



disease, suggesting that circulating miRs could provide information regarding the cardiac status.

Next step

(i). Increase the number of samples to confirm our findings.

(ii). To follow-up the volunteers who showed marked changes in the miRs. This will be done by routine blood sampling and echocardiography measurement of cardiac functions..

We are currently applying for additional funds to progress our research to the next step.

Conclusion: This study has demonstrated that measuring the circulating levels of cardiac specific miRs could become a valuable tool for the diagnosis of DHD, although the long-term follow up is required to confirm the results. Additional studies are on the way to confirm our findings and we are working towards publication of the results.

Publications

1. Published in Cardiovascular Diabetology 2014 Apr 1;13:68. doi: 10.1186/1475-2840-13-68. (IF – 4.2), marked as highly accessed publication.

Title - Rapid onset of cardiomyopathy in diabetic female mice involves the downregulation of pro-survival Pim-1 kinase

Authors - Andrew Moore, Amol Shindikar, Ingrid Fomison-Nurse, Federica Riu, Pankaj Saxena, Pujika Munasinghe, Thrishila Parshu Ram, Richard W. Bunton, Ivor F. Galvin, Costanza Emanueli, Paolo Madeddu, Rajesh Katare

Contribution from the grant – This study measured the gender-specific differences in the levels of microRNAs between diabetic and non-diabetic hearts.

2. Manuscript in preparation (Target journal – Diabetologia, IF - 6.5)

Title – Diabetes alters the correlation between circulating and tissue microRNAs

Authors – Thrishila Parshu Ram, Shruti Rawal Mahajan, Ingrid Formison-Nurse, Pujika Munasinghe, Richard W. Bunton, Ivor F. Galvin, Rajesh Katare

Contribution from the grant – This is a brief communication from the results described above regarding the correlation levels of microRNAs.

PART D – Dissemination and implementation of research results

1. Jono Paulin, Ingrid Fomison-Nurse, Pujika Munasinghe, Rajesh katare. Investigation of cardiac specific microRNA as novel biomarkers for diabetic

cardiomyopathy. Annual Scientific Session of the New Zealand Society for the Study of Diabetes, May 2013, Napier, New Zealand.

2. Andrew Moore, Amol Shindikar, Ingrid Formison-Nurse, Rajesh Katare. Gender differences in diabetic heart disease – the role of pro-survival proteins. Queenstown Research Week, August 25-29, 2013, Queenstown, New Zealand.

3. Ingrid Fomison-Nurse, Jono Paulin, Richard W. Bunton, Ivor F. Galvin, Rajesh Katare. Role of miR-34a in promoting ageing of the diabetic heart. Presenting author. Queenstown Research Week, August 25-29, 2013, Queenstown, New Zealand.

4. Thrishila Parshu Ram, Ingrid Fomison-Nurse, Pankaj Saxena, Ashvini Menon, Richard Bunton, Ivor Galvin, Rajesh Katare. Correlation of circulating and tissue levels of cardiac specific miR-1 and miR-208 in patients with cardiovascular disease. Presenting author. Queenstown Research Week, August 25-29, 2013, Queenstown, New Zealand.

5. Shruti Rawal, Patrick Manning, Rajesh Katare. Circulating microRNAs as biomarkers for early diagnosis of diabetic heart disease. Queenstown Research Week, August, 2014, Queenstown, New Zealand.

6. Katare R. Circulating microRNAs are early biomarkers for diabetic heart disease. Annual Scientific Session of Association of Physiologists of India, Key note lecture, December 2014, Pondicherry, India.

REFERENCES

1. Wild, S., et al., *Global prevalence of diabetes: estimates for the year 2000 and projections for 2030.* Diabetes Care, 2004. **27**(5): p. 1047-53.
2. Kannel, W.B., M. Hjortland, and W.P. Castelli, *Role of diabetes in congestive heart failure: the Framingham study.* Am J Cardiol, 1974. **34**(1): p. 29-34.
3. Booth, G.L., et al., *Relation between age and cardiovascular disease in men and women with diabetes compared with non-diabetic people: a population-based retrospective cohort study.* Lancet, 2006. **368**(9529): p. 29-36.
4. Cubbon, R.M., et al., *Temporal trends in mortality of patients with diabetes mellitus suffering acute myocardial infarction: a comparison of over 3000 patients between 1995 and 2003.* Eur Heart J, 2007. **28**(5): p. 540-5.
5. Mosterd, A., et al., *The prognosis of heart failure in the general population: The Rotterdam Study.* Eur Heart J, 2001. **22**(15): p. 1318-27.
6. Katare, R., et al., *Benfotiamine improves functional recovery of the infarcted heart via activation of pro-survival G6PD/Akt signaling pathway and modulation of neurohormonal response.* J Mol Cell Cardiol, 2010. **49**(4): p. 625-38.
7. Katare, R., et al., *Intravenous gene therapy with PIM-1 via a cardiotropic viral vector halts the progression of diabetic cardiomyopathy through promotion of prosurvival signaling.* Circ Res, 2011. **108**(10): p. 1238-51.
8. Katare, R.G., et al., *Vitamin B1 analog benfotiamine prevents diabetes-induced diastolic dysfunction and heart failure through Akt/Pim-1-mediated survival pathway.* Circ Heart Fail, 2010. **3**(2): p. 294-305.

9. Condorelli, G., M.V. Latronico, and G.W. Dorn, 2nd, *microRNAs in heart disease: putative novel therapeutic targets?* Eur Heart J, 2010. **31**(6): p. 649-58.
10. Depre, C., et al., *Streptozotocin-induced changes in cardiac gene expression in the absence of severe contractile dysfunction.* J Mol Cell Cardiol, 2000. **32**(6): p. 985-96.
11. Di Bonito, P., et al., *Early detection of diabetic cardiomyopathy: usefulness of tissue Doppler imaging.* Diabet Med, 2005. **22**(12): p. 1720-5.
12. Bonauer, A., R.A. Boon, and S. Dimmeler, *Vascular microRNAs.* Curr Drug Targets, 2010. **11**(8): p. 943-9.
13. Small, E.M. and E.N. Olson, *Pervasive roles of microRNAs in cardiovascular biology.* Nature, 2011. **469**(7330): p. 336-42.