miRNAs involved in neuropathic pain can be reliably measured in saliva

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Short Report

Keywords: miRNA, Neuropathic Pain, Saliva, Non-invasive, Diabetes

Posted Date: December 6th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3705352/v1

Abstract

Background: miRNAs have been shown to be dysregulated in Neuropathic pain conditions such as diabetic painful neuropathy (DPN). While invasive techniques such as blood samples are routinely used to collect and analyse miRNAs, the use of non-invasive techniques such as salivary samples for analysing miRNAs involved in neuropathic pain has been minimal. Therefore, the objective of this study was to determine if miRNAs involved in neuropathic pain can be measured reliably in salivary samples comprising of healthy European and Pacific population.

Methods: Participants were recruited via advertisements on notice boards, social media, word of mouth, and pamphlets. Saliva samples were collected from healthy European and Pacifica Participants. Samples were stored in -80ºC until analysis. Total RNA was extracted using miRNEasy kit (Qiagen) following manufacturer's protocol and the concentration was measured using Nanodrop (Thermofisher).

Results: A total of 37 healthy participants (19 European and 18 Pacifica; age range: 22-57 years) were included in the study. Results showed that four different miRNAs (miR-16, miR-124, miR-132 and miR-134) that have been demonstrated to be associated with DPN were expressed and reliably measured in all the salivary samples.

Conclusion: all the miRNAs identified in our study have been shown to be involved in neuropathic pain and inflammation. Hence, further research is required in this area to investigate the feasibility of extracting and analysing these miRNAs in people with neuropathic pain.

Introduction

According to the International Association of Study of Pain (IASP), neuropathic pain can be caused by a lesion or disease of the somatosensory system (Colloca et al., 2017), such as central nerve injury (e.g., stroke, multiple sclerosis, and spinal cord injury) and peripheral nerve injury (diabetes mellitus, peripheral nerve compression, and postherpetic neuralgia) (Scholz et al., 2019). At a molecular level, the neuro-immune changes in the nervous system as noted in people with neuropathic pain may result in altered regulation of gene expression (López-González, Landry, & Favereaux, 2017) with microRNAs (miRNAs) emerging as key regulators (Condorelli, Latronico, & Dorn, 2010; Rawal, Manning, & Katare, 2014). Specifically, miRNAs may regulate neuro-immune communication signals in the pain pathway by controlling macromolecular complexes in neurons, glia and immune cells. Neuropathic pain conditions such as diabetic painful neuropathy (DPN) has been associated with deregulated miRNA expression (Baron, Förster, & Binder, 2012; Shahar Barbash, 2012). Several studies have shown alterations in miRNAs in neuropathic pain in animal models (Liu et al., 2020; Sakai et al., 2013; Wang et al., 2018; W. Zhang, Zhou, & Zhang, 2022; Zhao et al., 2023). Recently, a scoping review identified and established miRNAs that are dysfunctional in people with neuropathic pain (Kovanur Sampath et al., 2023). The review further emphasised the use of non-invasive techniques such as salivary samples for collecting and analysing miRNAs for people with DPN. Recent studies have demonstrated the potential use of salivary miRNAs in diagnosis of cardiovascular disease(Al-Rawi, Al-Marzooq, Al-Nuaimi, Hachim, & Hamoudi, 2020; Khalyfa & Gozal, 2014; Monfared et al., 2021). This may facilitate the use of miRNAs as biomarkers of pain in future research studies. Therefore, the objective of this study was to determine if miRNAs involved in neuropathic pain can be measured reliably in salivary samples comprising of healthy European and Pacific population.

Methods

This study was approved by the University of Otago Human Ethics Committee (H22/017) and conformed to the standards set by the declaration of Helsinki. Due to potential of violating cultural sensitivities, Pacific communities were consulted for development of a culturally sensitive protocol regarding both the recruitment and collection of biological samples ensuring that this research was undertaken with the utmost respect for all PI peoples (Thompson et al., 2020). Participants were recruited via advertisements on notice boards, social media, word of mouth, and pamphlets. Following written consent, saliva samples were collected from healthy European and Pacifica Participants. Samples were stored in -80ºC until analysis. Total RNA was extracted using miRNEasy kit (Qiagen) following manufacturer's protocol and the concentration was measured using Nanodrop (Thermofisher). Twenty nanogram of total RNA was then reverse transcribed, followed by amplification using specific primers against miR-16, -124, 132 and – 134. miR-24 was used as the internal control (all primers from Thermofisher). For quantification, the amount of miRNA was normalized to the amount of miR-24 using the 2^{Δ}DCT method. Each reaction was performed in triplicate and repeated at least twice (repeated three times if duplicate results were inconsistent) (Katare et al., 2011). The primary outcome for this study is to determine if the neuropathic pain related miRNAs can be measured in Saliva and the secondary outcome is to determine if there is any difference in the expression of these miRNAs between healthy European and Pacifica populations. Difference between Eurpean and Pacific population for the expression of miRNAs were analysed using Graphpad prism. All the data failed Normality test (Shapiro-Wilk Test), hence the data were analysed using non-parametric Mann-Whitney test.

Results

A total of 37 healthy participants (19 European and 18 Pacifica; age range: 22–57 years) were included in the study. Results showed that four different miRNAs (miR-16, miR-124, miR-132 and miR-134) that have been demonstrated to be associated with DPN were expressed and reliably measured in all the salivary samples. However, our study did not show any significant difference in the expression pattern of salivary miRNAs between European and Pacifica population (refer Fig. 1).

**********INSERT FIGURE 1 HERE**********

Discussion

We are confident that the present study is the first of its kind to have identified and reliably measured salivary miRNAs such as miR-16, miR-124, miR-132 and miR-134 and have been shown to be dysfunctional in people with neuropathic pain (Andersen, Duroux, & Gazerani, 2014). Hence our findings are of extreme importance. Our recent review identified miR-132 in three out of the five studies to be an important miRNA involved in neuropathic pain. MiR-132 is abundantly expressed in the brain and spinal cord; is a key regulator of cognition, neuronal plasticity, and memory (Bredy, Lin, Wei, Baker-Andresen, & Mattick, 2011; Soreq & Wolf, 2011). MiR-132 has been implicated in neuropathic pain after chronic constriction injury (CCI) (Arai et al., 2013) and spared nerve injury (SNI) (R. Zhang et al., 2015). Crucially, a study found that miR-132-3p was significantly increased in WBCs of neuropathic pain patients compared to healthy controls, and that miR-132-3p expression in the sural nerve was correlated with pain intensity in patients with peripheral neuropathies(Leinders, Üçeyler, Pritchard, Sommer, & Sorkin, 2016).

miR-124 are also shown to be involved in the regulation of inflammatory processes in neuropathic pain. An increased expression of miR-124a enhanced CD41 T-cell differentiation in patients with neuropathic pain as compared with healthy volunteers(Luchting, Heyn, Hinske, & Azad, 2017). Evidence also demonstrates that miR-16 inhibits the expression of IL-1β and TNF-α, suggesting that miR-16 is involved in regulating inflammation in

neuropathic pain(Li et al., 2019). In a rat model of chronic sciatic nerve injury (CCI), miR-134-5p was shown to be significantly decreased. Importantly, overexpression of miR-134-5p alleviated neuropathic pain symptoms including mechanical and thermal hyperalgesia(Ji, Su, Xu, Pang, & Huang, 2019). In conclusion, our results show that salivary sample is a non-invasive and may be used as a reliable way to measure miRNAs involved in neuropathic pain.

Declarations

Disclosure of potential conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Assoc Prof Steve Tumilty for his inputs.

Source of Funding

This study was supported by a contestable research grant from the Waikato Institute of Technology, Te Pukenga, Hamilton, New Zealand.

Author Contributions

KSK conceived the study; RK and DOS drafted ethics application; ER recruited the participants, consented and collected saliva samples; JBP carried out all the PCR experiments and analysed the data; KSK wrote the initial draft of the manuscript; RK wrote the analysis section of the manuscript and provided edits on the draft. All authors read and approved the final manuscript.

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Figures

Figure 1:

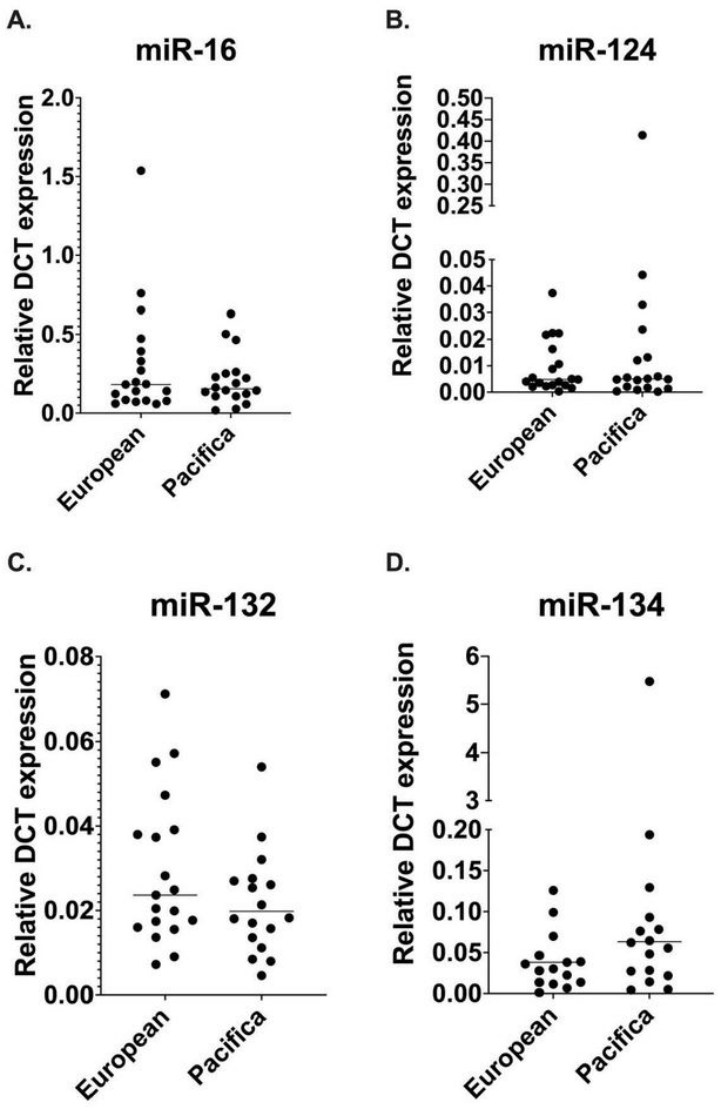

Figure 1

Quantitative scatter plots showing the expression levels of miR-16 (**A**), miR-124 (**B**), miR-132 (**C**), and miR-134 (**D**) in the saliva collected from healthy European (n=19) and Pacifica (n=18) participants. Comparisons between groups were calculated using the Mann-Whitney test.