

Abstract

Introduction: Vernal Keratoconjunctivitis (VKC) is an allergic inflammatory condition of the eye in children and young adults. MicroRNAs (miRNAs) are small non-coding RNA molecules which regulate various cellular processes in eye diseases. We aim to evaluate extracellular miRNAs in tears as the potential diagnostic biomarkers of VKC. **Methodology:** A case-controlled study was conducted at the Ophthalmology Clinic, Hospital USM and Molecular Genetics Lab, Universiti Sains Malaysia, Kelantan. MicroRNA microarray profiling was performed in four children with VKC and four controls.

Results: Out of 51 miRNAs, 48 were significantly up-regulated and three were significantly down-regulated in tears of children with VKC in comparison to controls. Among these, 37 miRNAs (35 up-regulated and two down-regulated) had Area Under Curve (AUC) score of 1.0, indicating possible candidates as diagnostic biomarkers for VKC. 12 novel miRNAs are observed in children with VKC. **Conclusion:** Study has provided definitive miRNA biomarkers which upon further validation would prove useful in VKC diagnosis and treatment.

Literature review

- VKC, Symptoms: Mucus discharge, severe itching, tearing, irritation and photophobia. Signs: Papillae, trantas dots, chemosis, and conjunctival hyperaemia.
- Epidemiological data strengthens genetic predisposition. Pathogenesis is complex.

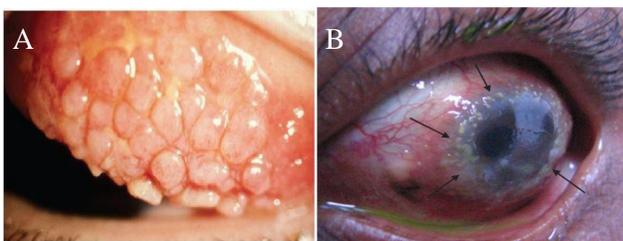


Figure 1: Cobble-stone papillae (A) and thick and opaque limbus showing Horner-Trantas dots (B).

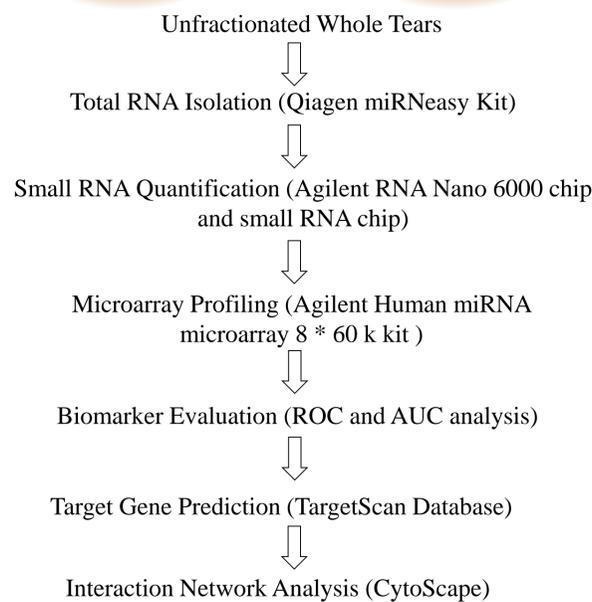
- MiRNAs play key role in ocular diseases like Sjögren syndrome, trachoma, retinoblastoma and pterygium.
- MiRNAs serve as diagnostic biomarkers for many diseases.
- MiRNAs regulate inflammation in allergic diseases.
- Tear fluid analysis is challenging due to its low volume
- MiRNA-profiling using microarray will reveal miRNAs expression pattern even at low sample volumes.

Methodology

- **Objectives:** To determine differential miRNA expression in tears of children with VKC and controls. To determine potential biomarkers and predicted target genes.
- **Ethical approval:** Human Research Ethics Committee of USM (USM/JEPeM/19090521)

Methodology

- A case-control study conducted at Ophthalmology Clinic, Hospital USM and Molecular Genetics Lab, Universiti Sains Malaysia, Kelantan, during January 2020 till January 2021.
- **Inclusion Criteria;** VKC group: 7-12 years old children with active symptoms of VKC, Control group: 7-12 years old children with no systemic and allergic diseases.
- **Exclusion criteria:** Children with symptoms and signs of any other allergic diseases and those on immuno-suppressive therapy.



Results

- Mean age of 10 years (VKC group) and 11 years (Control group).
- Distinct and low total RNA concentrations. However, miRNA concentrations were higher.
- 51 miRNA, 48 up-regulated and 3 down-regulated were significantly expressed after microarray analysis.

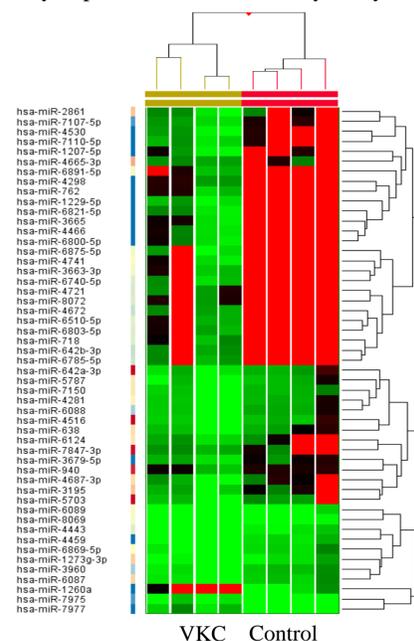


Figure 2: Clustered heat map of significantly expressed miRNAs in tears of children with VKC compared to control children. Moderated T-Test, $P \leq 0.05$ and $FC \geq 2.0$.

Results

- 48 up-regulated miRNAs have better AUC scores (≥ 0.875) than the AUC scores of down-regulated miRNAs (≥ 0.625).
- A total of 2290 target genes (TargetScan). Of these 16 genes, i.e., *PTGER*, *B3GAT1*, *SOCS3*, *ICOSLG*, *TGM2*, *MMP25*, *NGFR*, *FOXP3*, *HRH1*, *ARHGEF5*, *LGALS9*, *CCL22*, *CD276*, *PTGDS*, *MIF* and *PGF* were involved in conjunctivitis (AllerGAtlas).

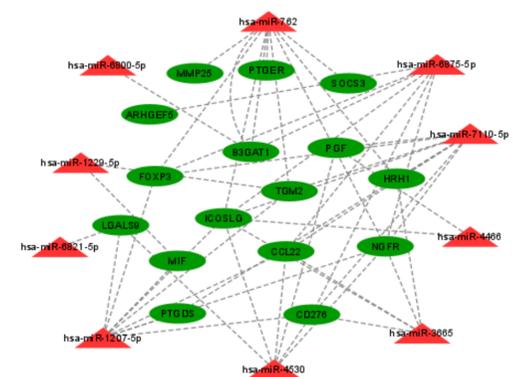


Figure 3: Illustrated network of miRNA and their target gene. The triangle nodes represent miRNA, and the oval nodes represent target genes.

Discussion

- Unlike the conventional usage of exosomes, unfractionated whole tears also had significant concentration of miRNA¹.
- The miR-4530 is reported to be involved in the regulation of inflammatory process, while miR-4672 is reported to targets genes related to innate immune system².
- In contrast to the findings of Liu et al., (2012), we have reported that miR-1260a is significantly down-regulated.
- System Development and Oxidative Phosphorylation were the top retrieved Gene Ontology term and KEGG pathway ID, respectively.
- The target genes were predicted to play role in inflammatory response through T_{reg} cells, NF-kappa and cytokine signaling pathways.
- **Conclusion:** This study has identified miRNAs that are present in children with VKC. Further validation and functional studies will help understand the pathogenesis and to design pharmaco-genetic treatment for the disease.

References

- ¹ Zhao, Lei, et al. "Isolation and Identification of miRNAs in exosomes derived from serum of colon cancer patients." *Journal of Cancer* 8.7 (2017): 1145.
- ² Pagliari, Matteo, et al. "Helicobacter pylori affects the antigen presentation activity of macrophages modulating the expression of the immune receptor CD300E through miR-4270." *Frontiers in immunology* 8 (2017): 1288.
- ³ Liu, Feng, et al. "Profiling of miRNAs in pediatric asthma: upregulation of miRNA-221 and miRNA-485-3p." *Molecular medicine reports* 6.5 (2012): 1178-1182.