Letter to the Editor

Plasma miR223 is a possible biomarker for diagnosing patients with severe atopic dermatitis

Dear Editor,

MicroRNAs (miRNA) are small non-coding RNAs built of 19–25 nucleotides, which bind to specific mRNAs to regulate translation or accelerate mRNA degradation.1 Recently some reported that there exist the close relationships between miRNAs and several allergic diseases such as asthma, allergic rhinitis, and atopic dermatitis (AD).2,3 In addition, miRNAs have been identified not only in cells but also in body fluids such as plasma, serum, and urine. It is thought that miRNAs in body fluids are very possible to be used as non-invasive biomarkers in detection and condition of diseases.

AD is a common chronic inflammatory skin disorder characterized by intense pruritus and recurrent eczematous lesions. It has been reported that some of the miRNAs are overexpressed in skin lesions, serum, and urine of AD patients and are involved in the pathogenesis of AD. It was identified that the miR-223 expressions in AD patients’ skin4 and whole blood cells4 were upregulated. However, there was no data about the miR-223 expressions of AD patients in body fluid and the relationship between the miR-223 levels and clinical data. In this study, we examined the miR-223 expressions in AD patients’ plasma, and the relationship between plasma miR-223 levels and the severity of AD or laboratory data.

A total of 21 adult patients (10 males, 11 females; median age: 43 years old; range: 22–62 years old) with AD were randomly selected and registered for this study. The patients were treated with conventional therapies, including topical corticosteroids and calcineurin inhibitors and systemic antihistamines. Patients that were taking systemic corticosteroids or immunosuppressive agents were not included in this study.

The severity of their AD was estimated using the eczema area and severity index (EASI) score (median score: 17.5; range: 0.4–50.5). Based on the EASI score, the 21 AD patients were divided into 3 groups: the mild (score: <10), moderate (score: 10–20), and severe (score: >20) groups (Supplementary Table 1, 2). The 10 urticarial patients (2 males, 8 females; median age: 38.5 years old; range: 23–61 years old) were randomly selected and registered for this study. The control group, 13 healthy volunteers (5 males, 8 females; median age: 36 years old; range: 22–54 years old) had not taken any medication for at least 2 weeks. All of the subjects provided written consent, and the study protocol was approved by the university ethics committee and was conducted in accordance with the Declaration of Helsinki. Detailed description of methods is provided in the Supplementary Methods online.

Fig. 1. Plasma miR223 levels of AD patients, urticarial patients and healthy volunteer.

A. The plasma miR223 expression levels of AD patients (n = 21), urticarial patients (n = 10), and healthy volunteer (HV) (n = 13) were measured. AD patients were divided into 3 groups based on their EASI scores; mild AD (score: <10) (n = 7), moderate AD (score: 10–20) (n = 7), and severe AD (score: >20) (n = 7). The Tukey–Kramer and Steel–Dwass tests were used to assess the significance of differences. Box plots presenting median, interquartile range, and maximum and minimum of the plasma miR223 expression levels are shown for all skin samples. *P < 0.05, **P < 0.01, Tukey–Kramer and *P < 0.05, **P < 0.01, Steel–Dwass tests.

B. The ROC curve establishing cut-off levels between patients with non-severe and severe AD for plasma miR223 expression levels (n = 21). AUC, Area under curve.

---

*Peer review under responsibility of Japanese Society of Allergology.

---

https://doi.org/10.1016/j.alit.2020.07.010
1323-8930 (Copyright © 2020, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
There was a significant difference regarding plasma miR223 expression levels among mild, moderate, and severe AD patients, urticarial patients, and healthy volunteers ($P < 0.01$). The severe AD patients’ plasma miR223 expression levels were significantly higher than those of mild AD patients ($P < 0.05$) and those of urticarial patients or healthy volunteers ($P < 0.05$) (Fig. 1A). The experiment using an independent replication dataset showed that plasma miR223 expression levels of severe AD patients were significantly elevated compared with those of urticarial patients or healthy volunteers ($P < 0.05$) (Supplementary Fig. 1). The receiver operating characteristic (ROC) curve establishing cut-off levels between patients with non-severe and severe AD for plasma miR223 expression levels is shown in Figure 1B. The area under the ROC curve was determined to be 0.837 (95% CI: 0.614, 1.00). This procedure was used to obtain a cut-off value, 4.85, giving the maximum efficiency in prediction of non-severe to severe AD with a sensitivity of 86% and a specificity of 93%. The apparent area under the ROC for the selected cut-off value of 4.85 was 0.65 (Supplementary Table 3).

The correlations between the plasma miR223 expression levels and laboratory data were evaluated. The results showed that miR223 expression levels were significantly correlated with the TARC ($r = 0.54$, $P = 0.042$), but not with the eosinophil counts ($r = 0.30$, $P = 0.286$), IgE ($r = 0.31$, $P = 0.239$), or LDH ($r = 0.49$, $P = 0.065$) (Fig. 2). These results indicate that plasma miR223 expression levels were significantly elevated in severe AD patients and were associated with serum levels of TARC, which stands for a suitable biomarker for monitoring AD severity. Therefore, miR223 levels seem to reflect to profound inflammation similar to TARC levels.

Histamine is one of the important inflammatory mediators in the pathomechanisms of allergic diseases, including AD. In mammals, histamine-N-methyltransferase (HNMT) is the major histamine degradation enzyme. Jin et al. have shown that the expression of HNMT was upregulated in both AD patients and AD

![Fig. 2. Correlation between the plasma miR223 level and serum TARC, IgE, LDH, or peripheral eosinophil in AD patients. The correlation between the plasma miR223 level and serum TARC ($n = 15$), IgE ($n = 15$), LDH ($n = 15$), or peripheral eosinophil counts ($n = 13$) was evaluated using Spearman’s rank correlation analysis.](image-url)
There was one miR223 binding site in the 3′-untranslated region of the HNMT gene. Therefore, miR223 might be possibly involved in the increase of HNMT to clear away the excessive histamine in AD patients.

Furthermore, Willeit et al. have reported that plasma miR223 levels are associated with platelet activation. In addition to their key functions in hemostasis and thrombosis, platelets are involved in the processes of inflammatory and immune responses. We have shown that platelets circulate in an activated state in AD patients, and the severity of AD is correlated with the degree of platelet activation. In addition, we have demonstrated that platelets play important roles in skin inflammation in AD model mice. These results suggest that miR223 may be related to the pathogenesis of AD through platelet activation.

In conclusion, this study showed that plasma miR223 levels were elevated in severe AD patients, and were related to serum TARC levels. These findings indicate that miR223 is significantly associated with the mechanisms of worsening atopic dermatitis, and indicate that plasma miR223 is a possible biomarker as diagnosis of severe AD.

Acknowledgements

We thank Hiromi Nishigaki for her technical assistance and Kei Fujikawa for his help with statistical analysis.

This work was supported in part by a grant from Torii Pharmaceutical Co., Ltd. and the Japanese Ministry of Education, Science, Sports, and Culture to R.T.M. and N.K.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2020.07.010.

Conflict of interest

The authors have no conflict of interest to declare.

References


Received 28 November 2019
Received in revised form 14 July 2020
Accepted 31 July 2020
Available online 1 September 2020

a These authors contributed equally to this work.