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MiR-185-5p measurement assists in reflecting Th1/Th2 cell imbalance, inflammatory cytokine production, and exacerbation risk for childhood asthma

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Abstract

Background: MicroRNA (miR)-185-5p participates in the pathology of asthma by regulating immune imbalance, inflammation, periostin synthesis, and smooth muscle contraction. This study intended to explore the dysregulation of miR-185p and its correlation with T-helper (Th)1, Th2 cells, and inflammatory cytokines in childhood asthma.

Methods: In 150 childhood asthma patients and 30 healthy controls (HCs), miR-185-5p from peripheral blood mononuclear cells was detected using reverse transcription-quantitative polymerase chain reaction, Th cells from peripheral blood samples were detected using flow cytometry, inflammatory cytokines from serum samples were detected using enzyme-linked immunosorbent assay.

Results: MiR-185-5p was increased in childhood asthma patients versus HCs [median (interquartile range (IQR)): 2.315 (1.770–3.855) versus 1.005 (0.655–1.520)] ($P < 0.001$). Meanwhile, miR-185-5p was negatively associated with Th1 cells ($P = 0.035$) but positively correlated with Th2 cells ($P = 0.006$) and IL-4 ($P = 0.003$) in childhood asthma patients; however, miR-185-5p was not linked to Th1 cells, Th2 cells, IFN- γ , or IL-4 in HCs (all $P > 0.05$). In addition, miR-185-5p was positively related to TNF- α ($P < 0.001$), IL-1 β ($P = 0.015$), and IL-6 ($P = 0.008$) in childhood asthma patients, miR-185-5p was only linked to TNF- α ($P = 0.040$) but not IL-1 β or IL-6 (both $P > 0.05$) in HCs. Moreover, miR-185-5p was increased in exacerbated childhood asthma patients versus remissive patients [median (IQR): 3.170 (2.070–4.905) versus 1.900

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(1.525–2.615)] ($P < 0.001$). Besides, miR-185-5p was highest in patients with severe exacerbation followed by patients with moderate exacerbation, and lowest in patients with mild exacerbation ($P = 0.010$).

Conclusion: MiR-185-5p is associated with imbalanced Th1/Th2 cells, increased inflammatory cytokines along with elevated exacerbation risk, and severity in childhood asthma patients.

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Introduction

Asthma is one of the most common respiratory diseases in children with symptoms such as coughing, wheezing, dyspnea, chest tightness, etc.¹ The management of childhood asthma includes pharmacological therapy (such as inhaled short-acting beta-2-agonists, inhaled steroids, leukotriene-modifying agents, etc.), bronchial thermoplasty, self-management, nutrition, etc.^{2–6} Unfortunately, childhood asthma cannot be completely cured, which may persist into adulthood.^{7,9} In addition, asthma could lead to severe asthma attacks, permanent decline in lung function, poor sleep, fatigue, etc., which are major causes of reduced quality-of-life and hospitalization in childhood asthma patients.^{10–13} Therefore, investigating potential biomarkers to better realize the stratified management of childhood asthma patients is crucial.

MicroRNA (miR)-185-5p plays a fundamental role in the regulation of asthma- pathophysiology-related biological processes, including inflammation and T helper (Th)1/Th2 cell balance.^{14–18} In detail, miR-185-5p regulates the secretion of inflammatory cytokines through cell division cycle 42 (CDC42)/c-Jun N-terminal kinase (JNK) pathway in macrophages.¹⁴ Moreover, miR-185-5p contributes to the imbalance of Th1/Th2 cells by regulating the mRNA ratio of T-bet/GATA-binding protein 3 (GATA-3).¹⁵ Notably, the role of miR-185-5p in the pathophysiology of asthma is also revealed by a study, which discovers that miR-185-5p regulates periostin synthesis and smooth muscle contraction to accelerate asthma.¹⁹ Based on the above evidence, it could be speculated that miR-185-5p may exert an essential clinical implication in childhood asthma patients. However, relevant evidence is scarce.

Accordingly, the current study intended to explore the dysregulation of miR-185-5p, and its association with Th1/Th2 cell imbalance, inflammatory cytokines, and asthma exacerbation in childhood asthma patients.

Materials and Methods

Subjects

From January 2021 to June 2022, a total of 150 childhood asthma patients and 30 healthy children were consecutively recruited for this case-control study. The inclusion criteria for patients with childhood asthma contained: (1) diagnosed as asthma per the guideline from Global Initiative for Asthma,²⁰ (2) aged ≤ 16 years, (3) without systemic autoimmune diseases, cancers, and hematologic malignancies. The healthy children were considered as

healthy controls (HCs) and the inclusion criteria contained: (1) ≤ 16 years old, (2) without any abnormalities in physical examinations, (3) without a history of asthma or allergic rhinitis. This study acquired permission from the Ethics Committee. Children or guardians signed the informed consent (if children were less than 8 years old, the signature was completed by the guardians only, otherwise the signature was completed by the children and guardians).

Data collection

Clinical characteristics were obtained for all subjects for study analysis, which included demographics, eosinophil count, immune globulin E (IgE), forced expiratory volume in 1 s (FEV₁), and forced vital capacity (FVC). In addition, a family history of asthma, history of allergic rhinitis and disease status were collected in patients with childhood asthma. Exacerbation severity was assessed in childhood asthma patients with exacerbating disease status as per the International Consensus on (ICON) Pediatric Asthma.²¹

Sample collection

Peripheral blood samples of all subjects were obtained after inclusion, then peripheral blood mononuclear cell (PBMC) and serum samples were isolated, respectively. The separated PBMC samples were adopted to detect miR-185-5p expression using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). A total of 108 subjects' peripheral blood samples were available to detect the proportion of T-helper (Th) 1 cell and Th2 cell by flow cytometry (FCM). Serum samples were adopted to detect the level of inflammatory cytokines by enzyme-linked immunosorbent assay (ELISA). The inflammatory cytokines included interferon-gamma (IFN- γ), interleukin (IL)-4, tumor necrosis factor-alpha (TNF- α), IL-1 β , and IL-6. The detection of IFN- γ and IL-4 was to verify the Th1/Th2 imbalance.²² Detection of TNF- α , IL-1 β , and IL-6 was due to miR-185-5p being reported to regulate TNF- α , IL-1 β , and IL-6 levels.¹⁴

Assay procedures

For RT-qPCR, RNeasy Protect Mini Kit (Qiagen, Germany) was utilized for total RNA extracting, PrimeScript RT Master Mix (Takara, China) was utilized for reverse transcription and SYBR® Premix DimerEraser (Takara, China) was utilized for qPCR. The miR-185-5p expression was evaluated by the $2^{-\Delta\Delta Ct}$ method, and U6 served

as the internal reference.²³ The primers were: miR-185-5p, forward: 5'-AATCGGCGTGGAGAGAAAGGC-3', reverse: 5'-GTCGTATCCAGTGCAGGGTCC-3'; U6, forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3', reverse: 5'-CGCTTCACGAATTTGCGTGCAT-3'. For ELISA, the commercial kits were purchased from Invitrogen Life Technologies, Inc. (USA) and utilized for detection, which included IFN- γ Human ELISA Kit (No. Cat. BMS228), IL-4 Human ELISA Kit (No. Cat. BMS225-2), TNF- α Human ELISA Kit (No. Cat. BMS202), IL-1 β Human ELISA Kit (No. Cat. KAC1211), and IL-6 Human ELISA Kit (No. Cat. KHC0061). For FCM, The Human Th1/Th2 Phenotyping Kit (Cat. No. 551810, BD, USA) was utilized. All procedures were in strict accordance with the instructions.

Statistics

Statistics were performed by SPSS V.24.0 (IBM Corp., USA). Figures were mapped by GraphPad Prism V.6.01 (GraphPad Software Inc., USA). Comparative analyses were completed using Student's t-test, χ^2 test, or Wilcoxon rank sum test. Association analyses were carried out using Spearman's rank correlation test. The differential effectiveness of miR-185-5p expression was evaluated using receiver-operating characteristic (ROC). Factors related to exacerbation were screened using Forward-stepwise logistic regression analysis. $P < 0.05$ were considered significant.

Results

Clinical features

The enrolled childhood asthma patients and HCs had a mean age of 7.4 ± 3.0 years and 8.2 ± 2.8 years ($P = 0.176$). Meanwhile, there were 78 (52.0%) female and 72 (48.0%) male childhood asthma patients, as well as 15 (50.0%) female and 15 (50.0%) male HCs ($P = 0.841$). In addition, height ($P = 0.063$) and weight ($P = 0.082$) were not different between childhood asthma patients and HCs. Furthermore, the median value of eosinophil count ($P < 0.001$) and IgE ($P < 0.001$) were increased but the mean value of FEV₁/FVC ($P < 0.001$) and FEV₁ ($P < 0.001$) were reduced in childhood asthma patients compared to HCs. Notably, Th1 cells ($P = 0.034$) and IFN- γ ($P < 0.001$) were reduced in childhood asthma patients compared with HCs while Th2 cells ($P = 0.003$), IL-4 ($P < 0.001$), TNF- α ($P < 0.001$), IL-1 β ($P < 0.001$), and IL-6 ($P < 0.001$) were raised in childhood asthma patients versus HCs (Table 1).

In addition, 69 (46.0%) childhood asthma patients had a status of remission, and 81 (54.0%) patients had a status of exacerbation. In exacerbated patients, 20 (24.7%) patients had mild exacerbation, 43 (53.1%) patients had moderate exacerbation, and 18 (22.2%) patients had severe exacerbation. The specific information is listed in Table 1.

MiR-185-5p expression

MiR-185-5p was increased in childhood asthma patients [median (IQR): 2.315 (1.770-3.855)] compared to HCs

[median (IQR): 1.005 (0.655-1.520)] ($P < 0.001$) (Figure 1A). Meanwhile, miR-185-5p had a good capacity to discriminate childhood asthma patients from HCs [area under the curve (AUC) (95% confidence interval (CI)): 0.894 (0.832-0.957)] (Figure 1B).

Correlation of miR-185-5p with Th1 cells, Th2 cells, and their corresponding cytokines

In childhood asthma patients, miR-185-5p was negatively related to Th1 cells ($r = -0.223$, $P = 0.035$) (Figure 2A) but positively associated with Th2 cells ($r = 0.285$, $P = 0.006$) (Figure 2B). In addition, miR-185-5p was not linked to IFN- γ ($r = -0.138$, $P = 0.093$) (Figure 2C) but positively correlated with IL-4 ($r = 0.239$, $P = 0.003$) (Figure 2D). In HCs, miR-185-5p was not associated with Th1 cells (Figure 2E), Th2 cells (Figure 2F), IFN- γ (Figure 2G), or IL-4 (Figure 2H) (all $P > 0.05$).

Association of miR-185-5p with proinflammatory cytokines

MiR-185-5p was positively related to TNF- α ($r = 0.284$, $P < 0.001$) (Figure 3A), IL-1 β ($r = 0.199$, $P = 0.015$) (Figure 3B), and IL-6 ($r = 0.215$, $P = 0.008$) (Figure 3C) in childhood asthma patients. However, miR-185-5p was only positively linked to TNF- α ($r = 0.377$, $P = 0.040$) (Figure 3D) but not associated with IL-1 β (Figure 3E) or IL-6 (Figure 3F) (both $P > 0.05$) in HCs.

Association of miR-185-5p with exacerbation severity

MiR-185-5p was increased in exacerbated childhood asthma patients [median (IQR): 3.170 (2.070-4.905)] in contrast to remissive patients [median (IQR): 1.900 (1.525-2.615)] ($P < 0.001$) (Figure 4A). Besides, miR-185-5p held a good ability to distinguish exacerbated childhood asthma patients from remissive patients [AUC (95% CI): 0.738 (0.660-0.816)] (Figure 4B). Moreover, miR-185-5p was highest in patients with severe exacerbation, followed by patients with moderate exacerbation and lowest in patients with mild exacerbation ($P = 0.010$) (Figure 4C).

Correlation of miR-185-5p with the level of FEV₁/FVC and FEV₁

The association of miR-185-5p with FEV₁/FVC and FEV₁ was also analyzed, and it was observed that miR-185-5p was negatively associated with both FEV₁/FVC ($r = -0.485$, $P < 0.001$) and FEV₁ ($r = -0.520$, $P < 0.001$) in childhood asthma patients.

Independent factors for exacerbation risk

Forward-stepwise logistic regression analysis revealed that higher miR-185-5p [odds ratio (OR) = 2.305, $P = 0.009$], IgE (OR = 1.018, $P < 0.001$), IL-4 (OR = 1.204, $P = 0.016$), TNF- α (OR = 1.043, $P = 0.007$), and IL-1 β (OR = 4.093, $P = 0.003$) all independently estimated increased exacerbation risk in childhood asthma patients (Table 2).

Table 1 Clinical characteristics.

Items	HCs (N = 30)	Childhood asthma (N = 150)	P value
Age (years), mean±SD	8.2 ± 2.8	7.4 ± 3.0	0.176
Gender, No. (%)	-	-	0.841
Female	15 (50.0)	78 (52.0)	-
Male	15 (50.0)	72 (48.0)	-
Height (cm), mean±SD	131.6 ± 18.8	125.0 ± 17.4	0.063
Weight (kg), mean±SD	30.0 ± 11.7	25.9 ± 9.5	0.082
Family history of asthma, No. (%)			
No	-	112 (74.7)	-
Yes	-	38 (25.3)	-
History of allergic rhinitis, No. (%)			
No	-	48 (32.0)	-
Yes	-	102 (68.0)	-
Disease status, No. (%)			
Remission	-	69 (46.0)	-
Exacerbation	-	81 (54.0)	-
Exacerbation severity*, No. (%)			
Mild	-	20 (24.7)	-
Moderate	-	43 (53.1)	-
Severe	-	18 (22.2)	-
Eosinophil count (10 ⁹ /L), median (IQR)	0.1 (0.1–0.1)	0.3 (0.2–0.5)	<0.001
IgE (IU/mL), median (IQR)	31.9 (24.5–57.1)	147.0 (81.7–268.4)	<0.001
FEV ₁ /FVC (%), mean±SD	84.8 ± 3.8	72.7 ± 7.7	<0.001
FEV ₁ (predicted) (%), mean±SD	98.7 ± 6.2	82.5 ± 8.1	<0.001
Th1 cells (%), median (IQR)	17.1 (13.3–23.3)	14.4 (11.2–17.3)	0.034
Th2 cells (%), median (IQR)	12.6 (10.6–14.4)	14.9 (12.4–18.1)	0.003
IFN-γ (pg/mL), median (IQR)	9.8 (8.7–14.4)	7.8 (6.1–10.8)	<0.001
IL-4 (pg/mL), median (IQR)	7.7 (5.8–9.8)	11.7 (8.4–15.2)	<0.001
TNF-α (pg/mL), median (IQR)	44.4 (37.4–54.1)	66.3 (50.4–85.7)	<0.001
IL-1β (pg/mL), median (IQR)	1.4 (1.1–1.8)	1.9 (1.5–2.6)	<0.001
IL-6 (pg/mL), median (IQR)	23.3 (18.7–31.1)	36.4 (26.7–45.2)	<0.001

*Exacerbation severity was evaluated among childhood asthma patients with disease status of exacerbation (n = 81).

HCs, healthy controls, SD, standard deviation, IQR, interquartile range, IgE, immunoglobulin E, FEV₁, forced expiratory volume in 1 second, FVC, forced vital capacity, Th, T-helper, IFN-γ, interferon-gamma, IL, interleukin, TNF-α, tumor necrosis factor-alpha.

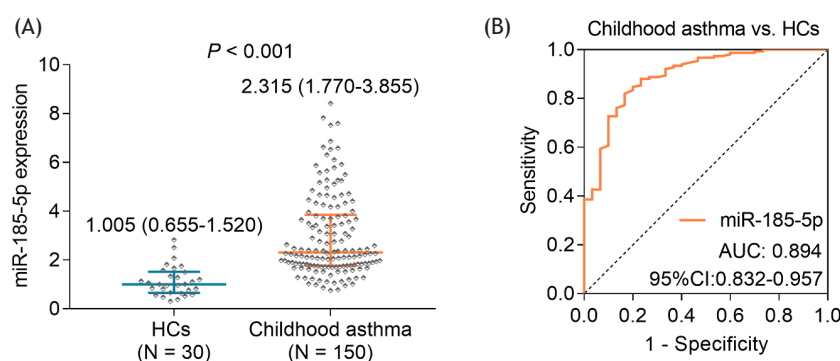


Figure 1 Comparison of miR-185-5p between childhood asthma patients and HCs. (A) MiR-185-5p was increased in childhood asthma patients versus HCs. (B) ROC curve for discriminating childhood asthma patients from HCs by miR-185-5p.

Discussion

The clinical importance of miR-185-5p in patients with respiratory diseases has been revealed by several previous studies.^{24,27} For instance, miR-185-5p exerts an acceptable ability to discriminate tuberculosis patients from HCs.²⁴ In addition, miR-185-5p is upregulated in asthma patients

compared to healthy subjects and possesses a good ability to discriminate asthma patients from healthy subjects.²⁶ However, the dysregulation of miR-185-5p in childhood asthma patients deserves further investigation. In line with this previous study,²⁶ the present study discovered that miR-185-5p was increased in childhood asthma patients compared to HCs; meanwhile, miR-185-5p had a good

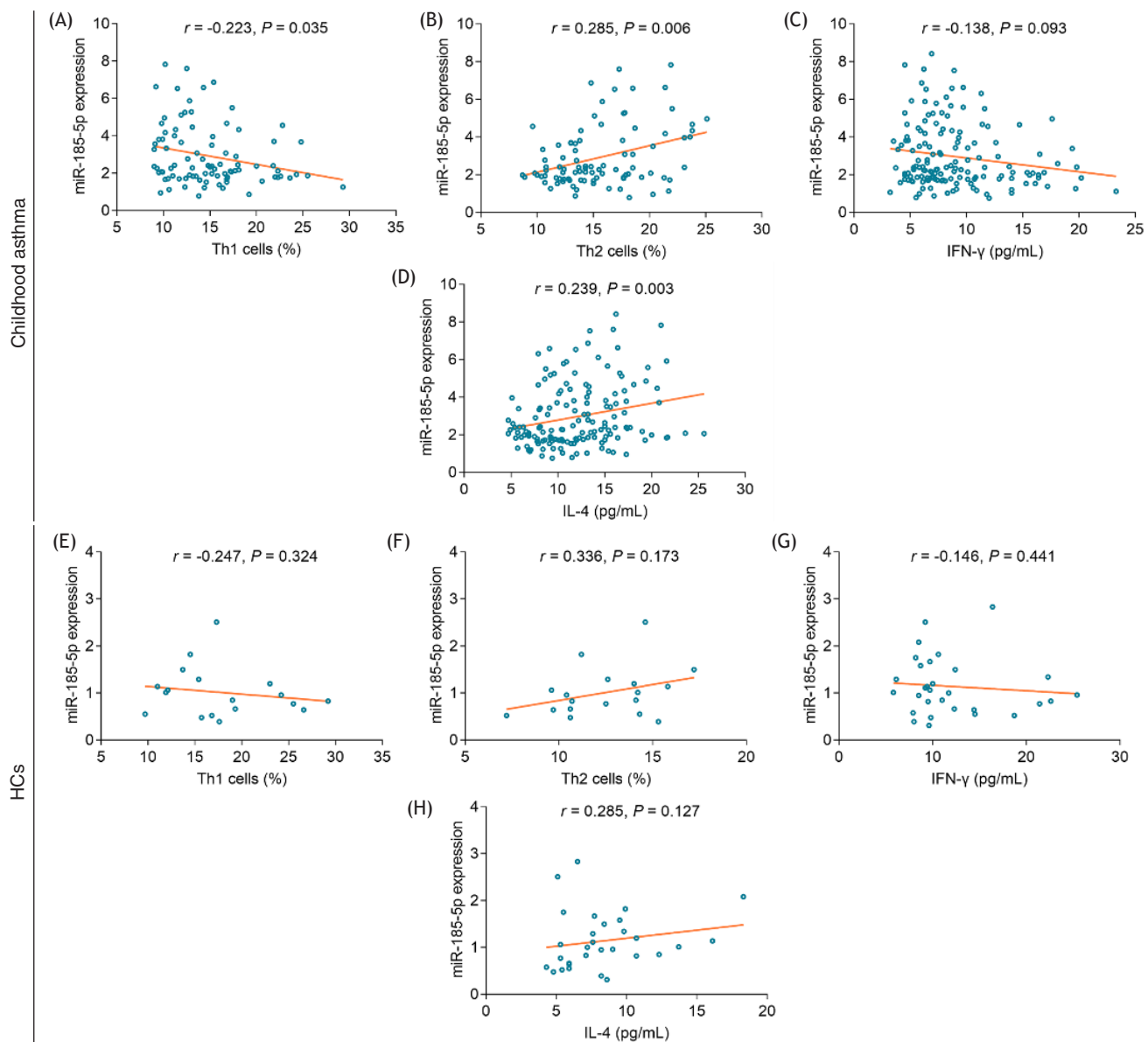


Figure 2 Association of miR-185-5p with Th1 and Th2 cells in childhood asthma patients and HCs. Relationship of miR-185-5p with (A) Th1 cells, (B) Th2 cells, (C) IFN- γ , and (D) IL-4 in childhood asthma patients, association of miR-185-5p with (E) Th1 cells, (F) Th2 cells, (G) IFN- γ , and (H) IL-4 in HCs.

ability to distinguish childhood asthma patients from HCs. The potential reasons would be as follows: (1) miR-185-5p could trigger the imbalance of Th1/Th2 cells and aggravate the inflammation, leading to the onset of childhood asthma^{14,15} and (2) miR-185-5p could also modulate the perostin synthesis and smooth muscle contraction to induce childhood asthma,¹⁹ thus, miR-185-5p could reflect higher childhood asthma susceptibility.

The association of miR-185-5p with Th1 and Th2 cells as well as their corresponding cytokines in childhood asthma patients was explored in the current study. It was observed that miR-185-5p was inversely related to Th1 cells but positively associated with Th2 cells in childhood asthma patients. A possible reason would be that miR-185-5p could elevate the mRNA ratio of T-bet/GATA-3, leading to the imbalance of Th1/Th2 cells,¹⁵ thus, a negative correlation

was observed between miR-185-5p and Th1 cells while a positive association was observed between miR-185-5p and Th2 cells in childhood asthma patients. In terms of the association of miR-185-5p with inflammation, the present study discovered that miR-185-5p was positively correlated with proinflammatory cytokines in childhood asthma patients. It could be explained by that miR-185-5p could facilitate the secretion of TNF- α , IL-1 β , and IL-6 in macrophages in various ways such as CDC42/JNK, sex-determining region Y-Box transcription factor 6, etc.^{14,28,29}, thus, miR-185-5p was positively linked to proinflammatory cytokines in childhood asthma patients.

Importantly, this study also disclosed that miR-185-5p was positively correlated with exacerbation severity in childhood asthma patients. The possible arguments would be that: (1) as discussed above, miR-185-5p could induce

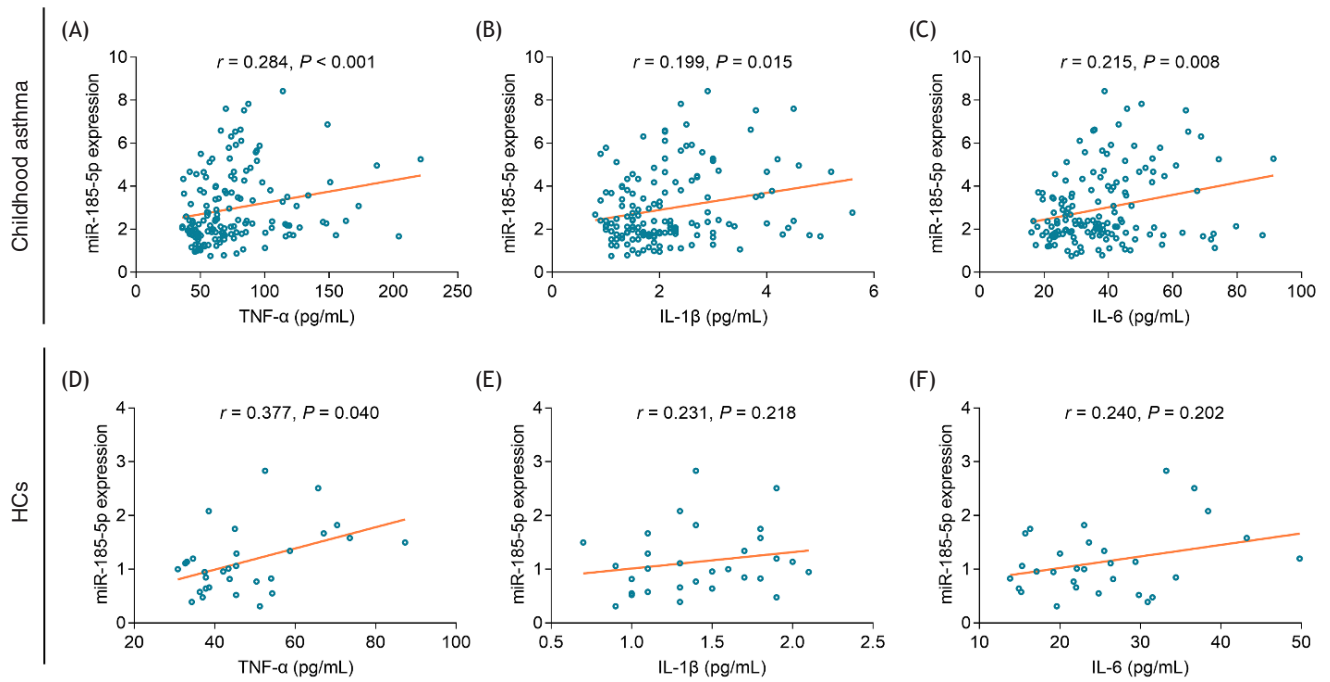


Figure 3 Relationship of miR-185-5p with proinflammatory cytokines in childhood asthma patients and HCs. (A) Association of miR-185-5p with TNF-α (B) IL-1β, and (C) IL-6 in childhood asthma patients, correlation of miR-185-5p with (D) TNF-α, (E) IL-1β, and (F) IL-6 in HCs.

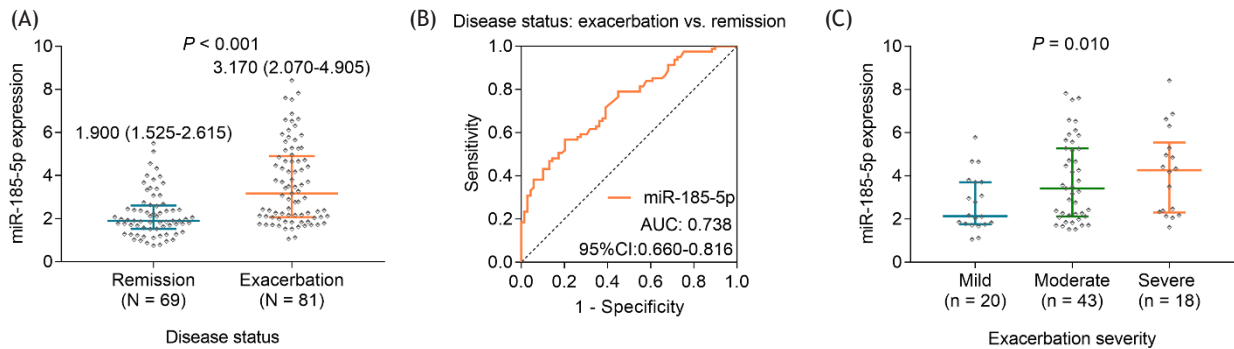


Figure 4 Relationship of miR-185-5p with exacerbation risk and severity in childhood asthma patients. (A) MiR-185-5p was increased in exacerbated childhood asthma patients compared to remissive patients. (B) ROC curve for discriminating exacerbated childhood asthma patients from HCs by miR-185-5p. (C) miR-185-5p was highest in patients with severe exacerbation, followed by patients with moderate exacerbation, and lowest in patients with mild exacerbation.

Table 2 Forward-stepwise logistic regression analysis for exacerbation risk.

Items	P value	OR	95% CI	
			Lower	Upper
Higher miR-185-5p expression	0.009	2.305	1.229	4.322
Higher IgE	<0.001	1.018	1.009	1.027
Higher IL-4	0.016	1.204	1.036	1.400
Higher TNF-α	0.007	1.043	1.012	1.076
Higher IL-1β	0.003	4.093	1.593	10.516

OR, odds ratio; CI, confidence interval; IgE, immunoglobulin E; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

immune imbalance and aggravate inflammation, contributing to the exacerbation of childhood asthma,^{14,15} (2) miR-185-5p might also modulate the protein secretion and smooth muscle contraction to aggravate childhood asthma,¹⁹ (3) miR-185-5p could regulate apoptosis and necroptosis in alveolar type II cells, which might further exacerbate lung damage.³⁰ Taken together, a positive association was observed between miR-185-5p and exacerbation severity in childhood asthma patients. Furthermore, miR-185-5p was also related to exacerbation risk in childhood asthma patients. In addition, miR-185-5p held a good ability to discriminate exacerbations from remissions. A potential reason would be that, as discussed above, miR-185-5p could contribute to aggravated inflammation, Th1/Th2 imbalance, lung damage, etc., hence, exacerbating

childhood asthma.^{14,15,19,30} As a result, miR-185-5p was correlated to exacerbation risk in childhood asthma patients.

Notably, miR-185-5p regulates periostin synthesis and smooth muscle contraction to participate in the pathology of asthma.¹⁷ Therefore, it seemed more pleasing to detect miR-185-5p in the respiratory epithelial cells. However, these cells were tough to collect. In addition, considering that asthma was mediated by inflammation, and the latter had a close correlation with lymphocyte cells. Therefore, detecting miR-185-5p from PBMC was representative and more feasible. In addition, the correlation coefficient was similar between HCs and childhood asthma patients. However, miR-185-5p was only related to Th1 cells, Th2 cells, and inflammatory cytokines in childhood asthma patients, but not in HCs. The possible reasons would be as follows: (1) the small sample size of HCs could affect the statistical power and further influence the results, therefore, the sample size of HCs should be expanded to validate these findings, (2) inflammatory status was stable in HCs, therefore, the dysregulation of miR-185-5p was controlled, which ultimately led to the correlation of miR-185-5p with Th1/Th2 cells and inflammatory cytokines were not obvious in HCs.

Several limitations should be noticed: (1) the clinical implication of miR-185-5p could be further explored in adult asthma patients, (2) multi-time detection of miR-185-5p was not realized in this study, which might be meaningful to investigate its role in monitoring disease progression for childhood asthma, (3) miR-185-5p was detected from PBMC in this study, while detecting miR-185-5p from respiratory lesions or saliva might be more representative for childhood asthma patients, but these samples were tough to collect, (4) considering that asthma was a lower respiratory disease, therefore, detecting miR-185-5p and cytokines from sputum might be also representative, (5) this was a case-control study; therefore, there was no washout period before enrollment, which might further affect the expression of miR-185-5p.

Conclusion

In summary, miR-185-5p exerts a potential ability to reflect higher disease risk, imbalanced Th1/Th2 cells, increased inflammatory cytokines, and elevated exacerbation risk and severity in childhood asthma patients. Clinically, the measurement of miR-185-5p may be valuable to assist in estimating the onset risk and exacerbation severity of childhood asthma. Whereas considering that this is the first study that explored the correlation of miR-185-5p with Th1/Th2 cells, inflammatory cytokines, and exacerbation risk in childhood asthma patients, further studies could expand the sample scale of patients to confirm these findings.

Conflict of Interest

The authors declare no conflict of interest.

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