Original Article
Diagnostic value of miR-221 and miR-142-3p expressions of allergic rhinitis, and miR-221 level is positively correlated with disease severity

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Abstract: This study was aimed to investigate the correlation of mast cells related microRNAs (miRNA) with risk of allergic rhinitis (AR) as well as disease severity. 85 patients with AR were recruited in this cross-sectional study, and 57 non-atopic patients with obstructive snoring undergoing adenoid surgery were also enrolled as controls. Nasal mucosa samples were collected from all participants and miRNAs were measured by Real-Time PCR methods. MicroRNA-123 (miR-126), miR-221, miR-222, miR-142-3p, miR-155, miR-132 and miR-143 were selected as candidate miRNAs base on screening of previous studies. Nasal mucosa miR-221 expression was elevated in AR patients compared to controls (1.989 (1.193-2.992) vs 1.575 (1.108-2.249), $P = 0.014$), as well as miR-142-3p level (3.355 (2.563-4.314) vs 1.982 (1.405-3.156), $P<0.001$). While no difference was found in other miRNAs between two groups. Receiver Operating Characteristic (ROC analysis) exhibited combination of miR-221 and miR-142-3p levels could predict the risk of AR with a high area under curve (AUC): 0.782, 95% CI: 0.707-0.858, as the sensitivity and specificity were 81.2% and 64.9% respectively at the best cut-off point, which is the point that has the best sensitivity and specificity. Additionally, AUC of individual miR-221 and miR-142-3p for AR were 0.622 (95% CI: 0.530-0.715) and 0.762 (95% CI: 0.683-0.841), respectively. Besides, miR-221 expression was illuminated to be positive correlated with total nasal symptom score (TNSS) ($P = 0.047$), itching score ($P = 0.011$) and sneezing score ($P = 0.022$). This study indicated nasal mucosa miR-221 and miR-142-3p expressions could be served as novel and promising biomarker for risk of AR, and miR-221 expression was associated with disease severity of AR.

Keywords: Allergic rhinitis, microRNA, risk, disease severity

Introduction

Allergic rhinitis (AR) is one of the multiple atopic disorders and mainly affects the nasal mucosa, which leads to nasal congestion, rhinorrhea, sneezing, and nasal itching [1]. AR displays a high morbidity among normal population, and appears more common in children and adolescents [1-3]. Due to various types of environmental allergens, early and late phase of hypersensitivity responses are motivated to mediate the pathogenesis of AR [1]. During the hypersensitivity responses, an amount of allergic inflammatory cells including mast cells, eosinophils and basophils are closely involved in the procedure [1].

Mast cells, derived from hematopoietic cells and exist in tissues as well as circulatory system, participate in atopic actions by secreting numbers of active materials as long life immune cells [4]. In AR responses, dendritic cells are stimulated by allergens, motivating the CD4^{+} T cells which play a crucial role in the process of hypersensitivity responses. Subsequently, IgE molecules are released and sensitize the mast cells. Cytoplasmic granule in mast cells synthesizes active mediators such as histamine, leukotrienes and prostaglandins, which resulted in multiple characteristic symptoms of AR [1]. In addition, the level of mast cells in nasal epithelial tissue and submucosal plexus is reported to be increased by 10 folds in allergic season [5].

MicroRNAs (miRNAs), a family of 21-25 nucleotide small RNAs, regulate gene expression via translational repression and/or mRNA degradation by binding to the 30 untranslated regions

Table 1. Demographic and clinical characteristics of AR patients and controls

Parameters	AR patients (n = 85)	Controls (n = 57)	p value
Age (years)	26±6	24±7	0.255
Female (%)	47 (55%)	28 (49%)	0.470
Disease duration (months)	94 (61-145)	-	-
INSS score			
Nasal rhinorrhea	2.00±0.66	-	-
Itching	1.91±0.55	-	-
Sneezing	2.42±0.65	-	-
Congestion	1.93±0.72	-	-
TNSS score	8.26±1.39	-	-

Data was presented as Mean values ± SD, median (25th-75th) or count (percentages). A p value <0.05 was considered statistically significant. Significance of the comparison is determined by the Student t test and the χ^2 test. INSS, Individual nasal symptom score; TNSS, Total nasal symptom score.

(30 UTRs) of their target mRNAs [6], which are reported to involved in allergic inflammation by mediating immune responses such as activating T cells, regulating eosinophil development and IL-13-driven epithelial responses [7]. And microRNA-146a (miR-146a) was found in nasal mucosa in mouse model and up regulate IL-10 expression which could suppress allergic reactions [8]. Several miRNAs have been discovered to be involved in the regulation of the mast cell activation, function, degranulation and differentiation in previous studies [6, 9-14], which contribute to the pathogenesis of AR. However, the role of mast cell related miRNAs in etiology of AR was obscure. Thus, this study was aimed to investigate the association of mast cell related miRNAs expressions in nasal mucosa with the risk and disease severity of AR.

Materials and methods

Participants

Eighty-five AR patients visited the Department of Otolaryngology of Renji Hospital were enrolled in this cross-sectional study between Oct. 2015 and Sep. 2016. The diagnosis of AR was based on the Allergic Rhinitis and Its Impact on Asthma (ARIA) guideline, which consists of the medical history, symptoms, and the presence of a positive skin x prick test. The major inclusion criteria were (1) patients who was diagnosed of AR; (2) age <60 years; (3) forced expiratory volume 1 (FaEV1) of the patients should be within the normal limit (>79% of predicted

value). Exclusion criteria were as follows: (1) patients with bronchial asthma, chronic rhino sinusitis, nasal polyposis, excessive septal deviation, and patients who were current smokers; (2) patients who received pharmacological treatment (antihistamines) within 10 days pre examination. And the study conducted a panel of common inhalant allergens among AR patients. In addition, fifty-seven age and gender matched non-atopic patients with obstructive snoring undergoing adenoid surgery were recruited as controls. Ethical approval was obtained from the Ethics Committee Boards of Renji hospital. Informed consents were acquired from all the participants.

Measurements of AR severity

To evaluate the severity of AR patients, the individual nasal symptom score (INSS) was used in this study, which consists of the scores of nasal rhinorrhea, sneezing, itching, and congestion individually. Each symptom was measured on a scale of 0-3 (0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms). Meanwhile, total nasal symptom score (TNSS) was also used in this study, which was the total score of nasal rhinorrhea, sneezing, itching, and congestion.

Samples

Nasal mucosa samples from the inferior turbinate of the AR patients and controls were obtained, and before the samples' collection local anaesthesia was performed on all patients. Samples were subsequently stored in liquid nitrogen under the temperature of -70°C.

Quantitative real-time PCR (qRT-PCR)

Total miRNA was extracted from each nasal mucosa sample using TRIzol regent (Invitrogen) according to the manufacturer's instructions. RNA was reversely transcribed by One Step Primer Script miRNA cDNA Synthesis Kit (Takara) afterwards. And the SYBR Premix Ex TaqTM II (Takara) was used to evaluate the quantity of candidate miRNAs, while the data of candidate miRNAs were normalized according to the expression of U6. After that, the results were calculated by 2-ΔΔt method.

miR-221 and miR-142-3p are associated with AR risk and severity
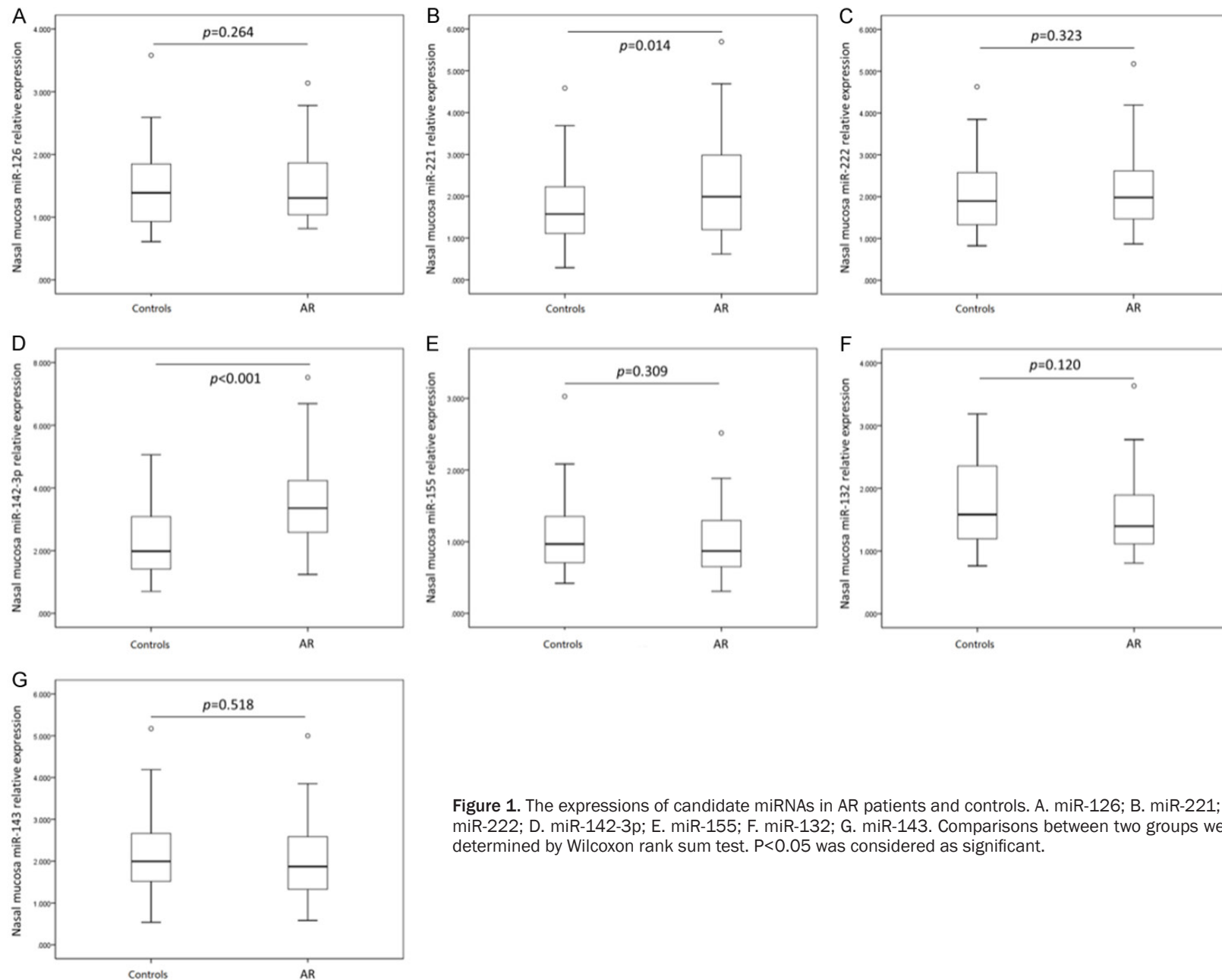
Figure 1. The expressions of candidate miRNAs in AR patients and controls. A. miR-126; B. miR-221; C. miR-222; D. miR-142-3p; E. miR-155; F. miR-132; G. miR-143. Comparisons between two groups were determined by Wilcoxon rank sum test. P<0.05 was considered as significant.
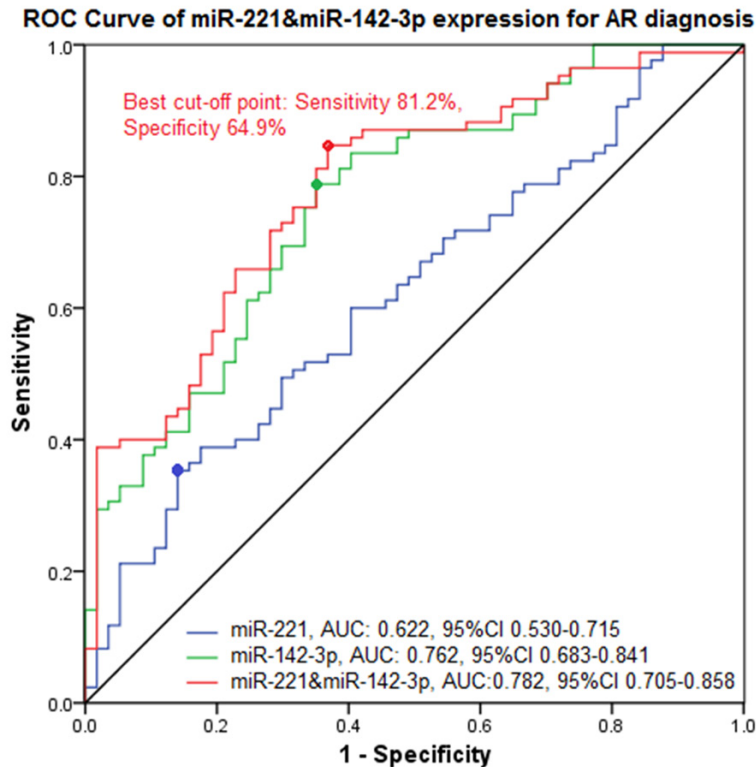
Figure 2. ROC curve of miR-221 and miR-142-3p expressions in AR diagnosis. The analysis was determined by ROC curve analysis. AUC of miR-221 was 0.622 (95% CI: 0.530-0.715) with specificity of 86.0%, sensitivity of 35.3%, PPV of 57.0% and NPV of 71.6% at best cut-off point, and the cut off value was 2.599. And AUC of miR-142-3p was 0.762 (95% CI: 0.683-0.841) with specificity of 64.9%, sensitivity of 78.8%, PPV of 69.1% and NPV of 75.3% at best cut-off point, and the cut off value was 2.440. When combing miR-221 and miR-142-3p, AUC was 0.782 (95% CI: 0.705-0.858) with the sensitivity of 81.2%, specificity of 64.9%, PPV of 69.8% and NPV of 77.5% at best cut-off point, and the cut off values of miR-221 and miR-142-3p were 1.446 and 2.368, respectively.

Student t test, Wilcoxon rank sum test or the χ^2 test between two groups, while Kruskal-Wallis H rank sum test was used to compare the difference among groups (≥3). Receiver operating characteristic curve (ROC) was drawn to evaluate the diagnostic value of candidate miRNAs in AR. A p value <0.05 was considered statistically significant.

Results

Participants

The overall demographic and clinical characteristics of AR patients (n = 85) and controls (n = 57) were listed in **Table 1**. No differences were observed in age as well as gender between AR patients and controls (P = 0.255 and P = 0.470, respectively). Among the AR patients, the disease duration of AR patients was 94 (61-145) months, and the TNSS score was 8.26±1.39, while individual INSS scores were as follows: nasal rhinorrhea 2.00±0.66, itching 1.91±0.55, sneezing 2.42± 0.65 and congestion 1.93± 0.72.

Selection of candidate miRNAs

Former studies characterizes that miR-126, miR-221, miR-222, miR-142-3p, miR-155, miR-132 and miR-143 are associated with proliferation, regulation of cell cycle and function of mast cells, which play critical role in pathogenesis of AR [6, 9-14]. Hence these miRNAs were chosen in this study to determine their expressions in order to explore their correlations with risk and severity of AR.

Statistics

Statistical analysis was performed by SPSS 21.0 (SPSS, Chicago, Illinois, USA). Data of age, gender, disease duration, INSS score, TNSS score and miRNAs was presented as Mean values ± SD, median (25th-75th) or count (percentage). The comparison was determined by the

The elevation of nasal mucosal miRNA expressions between AR patients and controls

Among the seven candidate miRNAs, the expression of nasal mucosal miR-221 in AR patients was increased compared with controls (1.989 (1.193-2.992) vs 1.575 (1.108-2.249), P = 0.014), as well as miR-142-3p level (3.355 (2.563-4.314) vs 1.982 (1.405-3.156), P<0.001) (**Figure 1**). While no differences were discovered between AR patients and controls in other remaining candidate miRNAs.

The diagnostic value of miRNA for AR

In order to further investigate the diagnostic value of miR-221 and miR-142-3p expression for AR, ROC curve was performed. The area under curve (AUC) of individual miR-221 and miR-142-3p for AR were 0.622 (95% CI: 0.530-
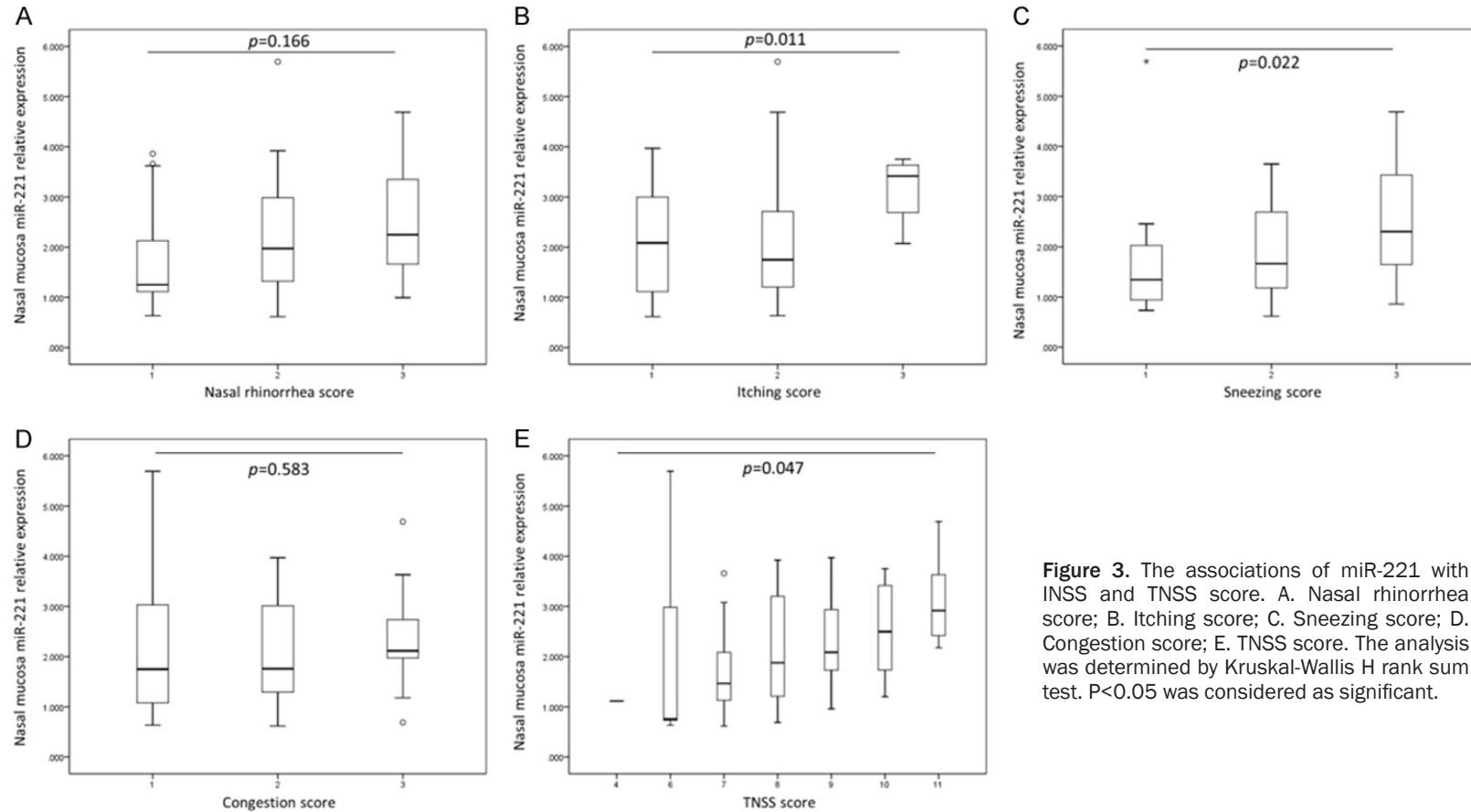
Figure 3. The associations of miR-221 with INSS and TNSS score. A. Nasal rhinorrhea score; B. Itching score; C. Sneezing score; D. Congestion score; E. TNSS score. The analysis was determined by Kruskal-Wallis H rank sum test. P<0.05 was considered as significant.

0.715) and 0.762 (95% CI: 0.683-0.841), respectively (**Figure 2**), which indicates that miR-221 and miR-142-3p could be good predictors for risk of AR separately. While combining the expressions of miR-221 with miR-142-3p, the AUC was 0.782 (95% CI: 0.705-0.858) with a sensitivity of 81.2% and a specificity of 64.9% at best cut off point (**Figure 2**), which suggests that the measurement of both miR-221 and miR-142-3p could be served as a strong diagnostic biomarker of AR.

The miR-221 level was positively correlated with disease severity of AR

Disease severity of AR patients was evaluated by INSS and TNSS score. To investigate the correlation of miR-221 and miR-142-3p expressions with disease severity of AR, Kruskal-Wallis H rank sum tests were performed between groups. The nasal mucosal miR-221 level was illustrated to be positively associated with TNSS score ($P = 0.047$), and correlated with itching score ($P = 0.011$) and sneezing score ($P = 0.022$), while no associations were found with nasal rhinorrhea score ($P = 0.166$) and congestion score ($P = 0.583$) (**Figure 3**). As to miR-142-3p level, no correlation was observed with nasal rhinorrhea score ($P = 0.337$), itching score ($P = 0.069$), congestion score ($P = 0.856$) and TNSS score ($P = 0.135$) but a positive correlation was discovered with sneezing score ($P = 0.039$) (**Figure 4**).

Discussion

Our study revealed that miR-221 and miR-142-3p expressions were elevated in nasal mucosa samples of AR patents compared with controls, and the combination of miR-221 and miR-142-3p levels exhibited a great value in diagnosis of AR with AUC 0.782 (95% CI: 0.705-0.858), as sensitivity and specificity were 81.2% and 64.9%, respectively. The correlations of miR-221 and miR-142-3p with AR severity were also analyzed and the results indicate that miR-221 was positively associated with TNSS score, which is a rating score of AR symptoms.

AR, as a seasonal inflammatory disorder, which burdens patients with numerous severe symptoms like sneezing and congestion, meanwhile AR is frequently ignored in clinical practice which results in unacceptable misdiagnosis and mistreatment [1, 15]. Recently, miRNAs

have been illustrated to get involved in the pathogenesis of AR in several ways such as regulation of mast cells and eosinophilic granulocytes. MiRNA-126 was reported to mediate mast cell proliferation by enhancing extracellular regulated protein kinases (ERK) and sprout-related, EVHI domain contmning 1 (spredl) expressions [12]. Mayoral RJ et al elucidated that miRNA-221-222 regulates mast cell proliferation cycle by suppressing stimulated cell cycle inhibitory protein p27^{kip1} [9]. Inhibition of miRNA-21 promotes eosinophilic granulocyte proliferation by targeting insulin-like growth factor I receptor (IGFIR) [16]. Wong CK et al discovered that up regulation of miRNA-21 expression mediates eosinophilic granulocyte apoptosis by enhancing granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulated ERK pathway [17]. In addition, Luo Y et al explored that up regulating miRNA-135a level decreased Th2 specific transcription factor GATA binding protein 3 (GATA-3) and IL-4 miRNA and protein, meanwhile increased T-bet and IFN-γ miRNA and protein, which corrected the Th1/Th2 unbalance in AR [18]. These indicate miRNAs play a crucial role in the etiology and development of AR.

MiR-221, belonging to miR-221/222 cluster, locates on chromosome Xp11.3 [19]. MiR-221 mediates mast cell degranulation, cytokine production and cell adherence in a mast cell-specific and activation dependent way [10]. Xu Hu et al illustrates that in a non-NF-κB dependent way, miR-221 stimulates IgE-translated activation of mast cell degranulation by PI3K/Akt/PLCγ/Ca(2+) pathway [20]. While mast cell functions as a crucial immunizing unit in immune responses of AR, thus the miR-221 plays an important role in AR through regulation of mast cell. In this present study, miR-221 was increased in AR patients compared with controls, and correlated with both risk and severity of AR, which is in accordance with the results in pediatric asthma patients [21].

MiR-142-3p, a hematopoietic specific miRNA [22], exists in multiple hematopoietic cells [23] and its target gene lies in the breakpoint junction of a t (8; 17) translocation [24]. MiR-142-3p overexpression reinforces the FcεRI-mediated degranulation of mast cells and rescued the decline of degranulation by silencing Dicer [6]. And miR-142-3p suppressed macrophage differentiation through down regulated gp130
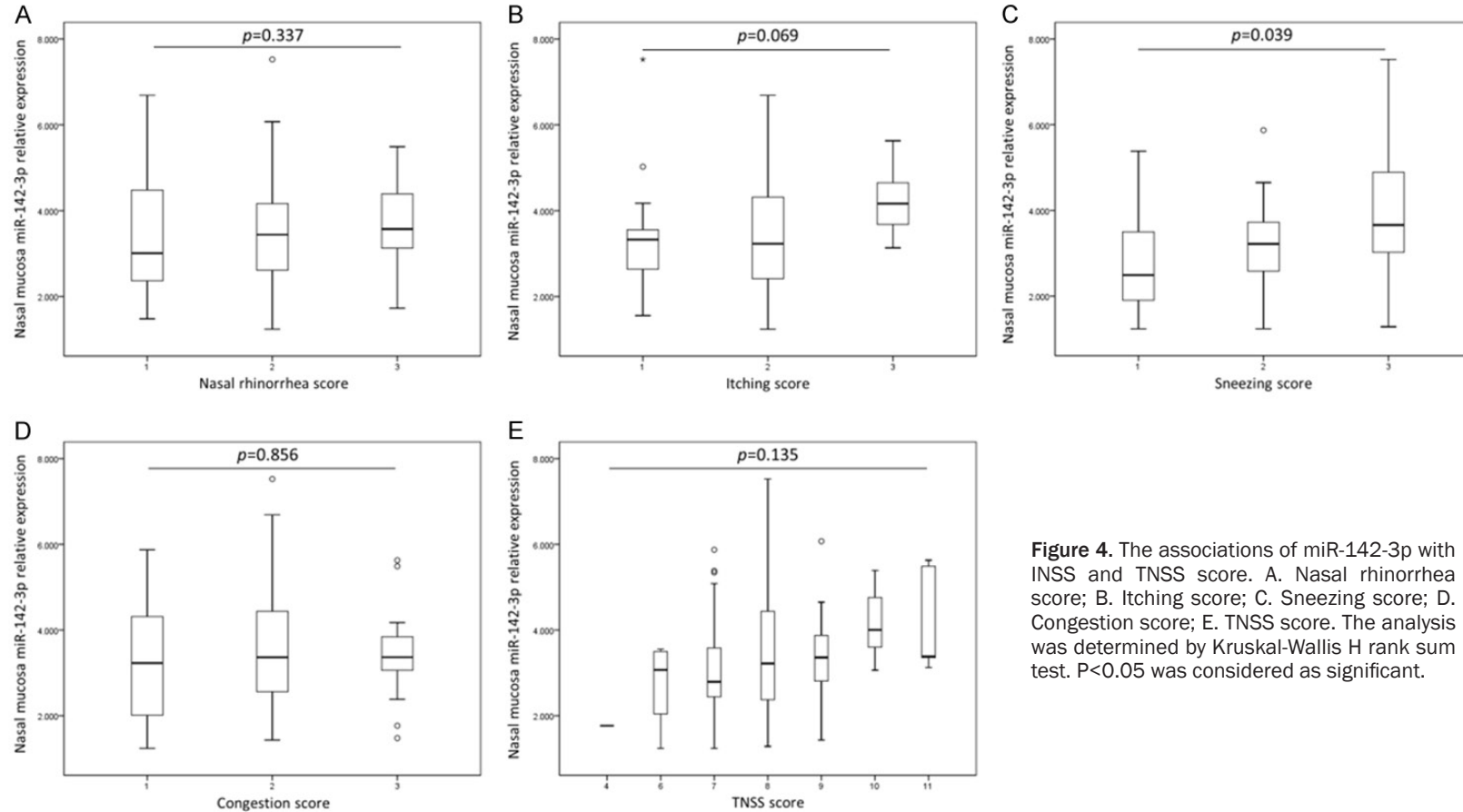
Figure 4. The associations of miR-142-3p with INSS and TNSS score. A. Nasal rhinorrhea score; B. Itching score; C. Sneezing score; D. Congestion score; E. TNSS score. The analysis was determined by Kruskal-Wallis H rank sum test. P<0.05 was considered as significant.

and CCAAT/enhancer-binding protein β (C/EBPβ) LAP [25]. In our study, miR-142-3p was found to be raised in AR patients compared with controls but not associated with disease severity.

In addition, we found ROC curve illuminated the combination of miR-221 and miR-142-3p expressions could predict AR risk with a good sensitivity and specificity (81.2% and 64.9%, respectively). And no similar research has illustrated the correlations of miR-221 and miR-142-3p with AR, which means the associations were first reported in this study.

Some limitations existed in this study: (1) This case control study enrolled 57 controls who were patients with obstructive snoring undergoing adenoid surgery instead of health controls, and it resulted from nasal mucosa samples were difficult and inappropriate to be obtained from healthy participants. Besides, obstructive snoring doesn't share the same mechanism with AR, and it reduced the bias in this study. (2) Our study was conducted in a single center, which indicated the results might not be as convincing as a multicenter study. (3) The sample size of this study was small with 85 AR patients and 57 controls which needed being enlarged in the future study.

This study indicated nasal mucosa miR-221 and miR-142-3p expressions could be served as novel and promising biomarker for risk of AR, and miR-221 expression was associated with disease severity of AR.

Disclosure of conflict of interest

None.

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