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Original Research

Serum levels of IL-31, IL-33 and ST2 in allergic rhinitis of children in China

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Abstract: This study aimed to compare the serum levels of IL-31, IL-33 and ST2 in children patients with allergic rhinitis (AR) and allergic asthma and tried to reveal more insights in development of AR. Thirty-six children patients with intermittent AR were selected in the present study during January 2013 to December 2016. Two control groups were set, the allergic asthma group and the healthy individuals. Atopic status of all participants in this study was tested and confirmed by skin prick testing (SPT). Levels of IL-31, IL-33 and ST2 were determined using ELISA. Statistical analysis was performed by SPSS 18.0. There is no significant difference in age, gender ratio and BMI for all patients. However, positive ratio of SPT and total IgE in patients' groups were significantly higher than the control group. Levels of IL-33 and IL-31 in both AR and allergic asthma group were significantly higher than those of the control group, $P<0.05$. However, level of ST2 in the three groups didn't show significant difference. Correlation analysis showed that there was a positive correlation between levels of IL-33 and IL-31, $P<0.05$. However, levels of ST2 and IL-33, ST2 and IL-31 were not significantly correlated. The serum levels of IL-33 and IL-31 were significantly up-regulated in both AR and allergic asthma patients compared with normal individuals. These results could provide more clinical evidences for understanding roles of IL-33, IL-31 and ST2 in children patients with allergic rhinitis.

Key words: IL-33; IL-31; Allergic rhinitis.

Introduction

Allergic rhinitis (AR), which is considered to be a nasal mucosa multifactorial inflammatory disorder, has affected the life quality of 10-30% adults and 10-46% children all over the world (1). It is characterized with the clinical symptoms of nasal, sneezing, and rhinorrhea, which can also influence function of bone marrow, peripheral blood, and even lungs (2). Studies show that that during last 30-40 years, the prevalence of AR has gradually increased during last 30-40 years, , especially in the industrialized countries (3).

Generally, it is thought that AR is a Th2 cell-mediated disease which is caused by the imbalance between Th1/Th2 immune response, leading to selective eosinophil accumulation in the nasal mucosa and allergen-specific immunoglobulin production (4). Several cytokines are involved in this process, such as interleukin IL-5, IL-13 and IL-18 (5). Recently, some new inflammatory factors are found to be associated with allergic diseases, in which IL-33 and IL-31 attracted people's eyes.

IL-33 is produced by mast cells after the process of immunoglobulin (Ig) E-mediated activation. IL-33 can lead to the release of proinflammatory cytokines by mast cells *in vitro*, suggesting that this cytokine plays a crucial role in allergic diseases (5-7). IL-33 can also induce polarization of Th cells, mast cells, basophils and eosinophils and so on, leading to stimulation of the production of proinflmmatory Th-related cytokines, such as IL-4, and IL-13 (8, 9). There are 2 receptors which can bind to IL-33, ST2 (IL-1R1) with 2 isoforms, ST2L is a transmembrane form and soluble ST2 (sST2) is a secreted form, and also IL-1 receptor accessory protein (IL-1RAP) (10, 11).

Studies showed that the IL-33/ST2 signaling could activate airway eosinophils and contribute to airway inflammation (12). It was found that ST2 was over-expressed on mast cells and was selectively on Th2 cells (13, 14). It was also found that in patients with asthma, sST2 level was up-regulated (15). Recently it is also reported that IL-33/ST2 signalling pathway is associated in AR (16).

Similarly, IL-31, mainly produced by activated Th cells, plays a key role in the development of atopic and allergic diseases. It has been proved that serum level of IL-31 significantly increased in patients with atopic dermatitis(17). Study also showed that IL-31 was related to allergic disease (18).

Though several studies have demonstrated the role of IL-33 and IL-31 in allergic disease, studies focusing on IL-33 and IL-31 in allergic rhinitis of children are still very few. In this study, we aimed to compare the serum levels of IL-33, IL-31 and ST2 in children patients with AR and allergic asthma. These results could provide more clinical evidences for understanding roles of IL-33, IL-31 and ST2 in children patients with allergic rhinitis.

Materials and Methods

Patients

In the present study, 36 of children patients with intermittent AR were selected with a mean age of 11.24±4.26 (7-14) years old during January 2013 to

December 2016. Two control groups were set, one was the allergic asthma group with 30 patients whose mean age was 11.83±3.81 ranging from 8-13 years old and the other was 30 healthy individuals whose mean age was 10.95±4.12 ranging from 7-13 years old.

The diagnosis of AR was according to Allergic Rhinitis and its Impact on Asthma guidelines (19, 20) and the diagnosis of allergic asthma was according to Global Initiative for Asthma guidelines (21, 22). Patients who had other serious chronic diseases, such as renal, cardiac or gastrointestinal diseases, and patients with any acute inflammatory diseases were excluded (23). Within 3 months before the study, no patient receives any treatment for asthma inhaled corticosteroids steroids (ICS), rhinitis antihistamines or topical steroids. All participants in the healthy control group had no any sign of allergic diseases or inflammatory diseases. Written informed consent was obtained from all participants and their parents. This study was approved by Ethics committee of the Shanghai Children's Medical Center Affiliated to the Medical School of Shanghai Jiaotong University.

Skin prick tests

All participants in this study were tested for atopic status by clinical history confirmed by skin prick testing (SPT) to one or more common aeroallergens according to EAACI guidelines (23, 24).

ELISA

Fasting cubital vein blood samples were collected from all participants. Levels of IL-33/ST2, IL-31 and total IgE were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems; Minneapolis, MN, USA). All procedures were in accordance with the manufacturer's instructions. Absorbance was assessed at 450 nm by a multi-detection microplate reader (Bio-rad, California, USA).

Statistical analysis

Data were expressed by mean ± SD **or** median values with interquartile ranges. Chi square test was used to analyze counting material. Kruskal-Wallis test was used to compare levels of IL-33, IL-31 and ST2 and Mann-Whitney U test was used to compare difference between two groups. Correlation statistical analysis was performed by Spearman rank test. All analyses were conducted using SPSS 18.0. *P* values less than 0.05 were considered significant.

Results

Clinical baseline of the patients

As shown in Table 1, no significant difference was observed in age, gender ratio and BMI. However positive ratio of SPT and total IgE in patients' groups were significantly higher than the control group.

Levels of IL-33, IL-31 and ST2 in different groups

As shown in Figure 1, levels of IL-33 and IL-31 in both AR and allergic asthma group were significantly higher than the control group, P<0.05. However, level of ST2 in the three groups didn't show significant difference.

Correlation analysis of IL-33, IL-31 and ST2 in different groups

Correlation analysis showed that there was a positive correlation between levels of IL-33 and IL-31 in both AR and allergic asthma group, P<0.05 (Figure 2). However, levels of ST2 and IL-33, ST2 and IL-31 were not significantly correlated (data not shown).

Discussion

As new found cytokines, IL-33 and IL-31 show biological functions in various of inflammations (17, 25, 26). Recently, scholars turned their eyes to roles of

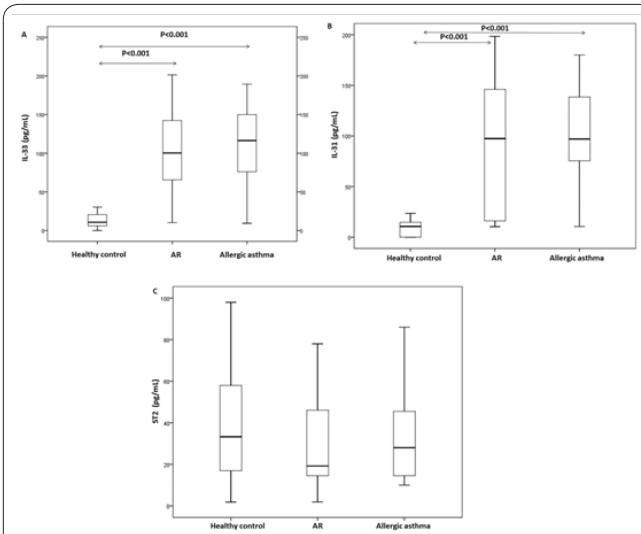

Figure 1. Levels of IL-33, IL-31 and ST2 in different groups.

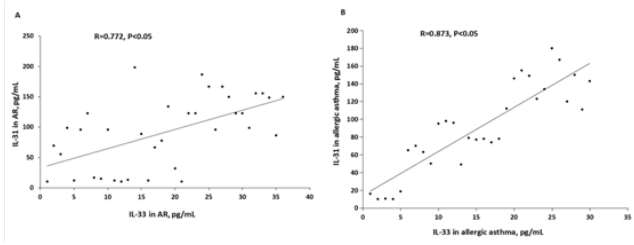

Figure 2. Correlation analysis of IL-33, IL-31 in AR and allergic asthma group.

Table 1. Clinical baseline of the patients.

	AR	Allergic asthma	Healthy control	*P* value
Subjects, n	36	30	30	-
Mean age, years	11.24±4.26 (7-14)	11.83±3.81 (8-13)	10.95±4.12(7-13)	0.647
Gender, male : female	22:14	14:16	17:13	0.885
BMI, kg/m^2	24.65±5.45	25.78±4.32	26.09±4.77	0.741
SPT positive ratio (%)	36 (100%)	30 (100%)	0 (0%)	0.000
Total IgE, IU/ml	327 (136~1098)	312 (165~1124)	45 (5~110)	0.000

IL-33 and IL-31 in allergic rhinitis.

Despite studies about IL-33 and IL-31 in allergic disease, studies focusing on IL-33 and IL-31 in allergic rhinitis of children are still very few. Thus the present study aimed to compare the serum levels of IL-33, IL-31 and ST2 in children patients with AR and allergic asthma and tried to reveal more insights in development of AR.

First we compared the clearly baseline of different groups and found no significant difference was observed in age, gender ratio and BMI. However positive ratio of SPT and total IgE in patients' groups were significantly higher than the control group. Than after comparison of levels of IL-33, IL-31 and ST2 by ELISA, we found that in both AR and allergic asthma group, IL-33 and IL-31 were significant higher than the control group. And levels of the two factors were statistically correlated. However, level of ST2 in the three groups didn't show significant difference and levels of ST2 and IL-33, ST2 and IL-31 were not significantly correlated. These results indicated IL-33 and IL-31 had the potential to be used as biomarkers for allergic rhinitis patients.

Several similar studies have been conducted previously. In 2012, study by Glück et al showed that serum IL-33 but not ST2 level was elevated in intermittent allergic rhinitis (16). Than Vocca et al studied relationship among IL-33, IL-31 and ST2 in patients with allergic airway diseases and found that plasma levels of IL-33 and IL-31 were significantly higher and sST2 was lower in patients with AR and concomitant allergic asthma and rhinitis than healthy individuals (27). However in Vocca's study sST2 was correlated with IL-33 and IL-31 which was not observed in the present study. The difference may be due to the different patients and sample size of the different studies.

Bonanno et al demonstrated that IL-31 and IL-33 plasma levels significantly increased in patients with concomitant allergic asthma and rhinitis than normal person and IL-31 and IL-33 positively correlated in AR and concomitant allergic asthma and rhinitis (18). I*n vitro* study also demonstrated similar results (28). All these studies are in consistent with the present study.

In conclusion, we conducted a study to compare the serum levels of IL-33, IL-31 and ST2 in children patients with AR and allergic asthma and found that levels of IL-33 and IL-31 were significantly up-regulated in both AR and allergic asthma patients compared with normal individuals. These results could provide more clinical evidences for understanding roles of IL-33, IL-31 and ST2 in children patients with allergic rhinitis.

Ethics statement

The Research Ethics Committee of Shanghai Children's Medical Center Affiliated to the Medical School of Shanghai Jiaotong University approved the collection of tissue samples for research.

Consent to publish

All of the authors have Consented to publish this research.

Authors' contributions

Yi Qiao for bioinformatics analysis and writing of the manuscript. Jie Chen for the discussion. Yi Qiao and Jie Chen for discussion and comments on an earlier version of the manuscript.

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Conflicts of interest

All authors declare no conflict of interest.

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