

# Correlation between asthmatic infants with rickets and vitamin D, inflammatory factors and immunoglobulin E

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**Abstract.** Correlation between asthmatic infants with rickets and vitamin D, inflammatory factors and immunoglobulin was investigated. A total of 60 child patients with asthma who met the inclusion criteria and received treatment from January 2016 to October 2017 were collected. Among them, 17 asthmatic infants with rickets were set as observation group, while 43 child patients with simple asthma were regarded as the control group. Venous blood was drawn from the two groups of subjects after admission. The levels of interleukin-1 (IL-1), IL-6 and IL-17 in serum were determined by ELISA, vitamin D and immunoglobulin E levels in serum were detected using a fully-automatic biochemical analyzer, and wheezing duration during asthma attack was recorded. IL-1, IL-6, IL-17 and immunoglobulin E levels in serum of observation group were significantly higher than those of the control group ( $P < 0.05$ ). The vitamin D level in the observation group was remarkably lower than that in the control group ( $P < 0.05$ ). Wheezing duration in observation group was evidently longer than that in control group ( $P < 0.05$ ). Moreover, IL-1, IL-6, IL-17 and immunoglobulin E levels in serum were positively related to wheezing duration, but the vitamin D level was negatively associated with wheezing duration. Infantile asthma with rickets is closely correlated with vitamin D, inflammatory factors and immunoglobulin E, which are major risk factors in infantile asthma with rickets.

## Introduction

Bronchial asthma is a clinically common disease in the Respiratory Department. It is an airway inflammatory disease characterized by airway hyperreactivity and increased secretion of airway mucus. This disease is closely related to tissue

inflammation caused by massive inflammatory cell infiltration (1). According to epidemiological statistics, asthma tends to occur in children, putting a heavy burden on patients, families and society (2). Moreover, it is proved that asthmatic infants with rickets account for about 20.1% of asthmatic infants in clinical practice (3). Vitamin D deficiency, as the main cause of infant rickets, can also lead to the imbalance of immunomodulatory mechanism in infants and young children, affecting the inherent immune system and acquired immune system, thus causing the attack of asthma (4). Vitamin D is positively correlated with the incidence of infection, which is the main cause of respiratory tract infection in children with rickets, and repeated respiratory tract infection can lead to increased airway responsiveness, which leads to asthma (5). Vitamin D not only adjusts calcium and phosphorus metabolism, but also plays an important role in cell growth, differentiation and immune function. Studies have shown that vitamin D deficiency can lead to B cell differentiation and maturation disorder, which in turn leads to hypoglobulin (6,7). There is a certain correlation between vitamin D, inflammatory factors and immunoglobulin in infants with asthma complicated with rickets.

This study investigated the correlation between asthmatic infants with rickets and vitamin D, inflammatory factors and immunoglobulin, so as to identify the risk factors of infantile asthma with rickets.

## Patients and methods

*General data.* A total of 60 children diagnosed as asthma and treated for the first time from January 2016 to October 2017 in The First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) were collected. Among them, according to the diagnostic criteria of rickets, 17 asthmatic infants with rickets were set as the observation group, while 43 children with simple asthma were regarded as the control group. There were 10 males and 7 females aged  $1.23 \pm 1.52$  years in the observation group, while there were 28 males and 15 females aged  $1.83 \pm 1.21$  years in the control group. No differences were found in age and gender between the two groups, which were comparable.

The study was approved by the Ethics Committee of The First Affiliated Hospital of Xinjiang Medical University and written informed consents were signed by the guardians.

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**Diagnostic criteria.** Diagnostic criteria of infantile asthma (8): i) age  $\leq 3$  years; ii) wheeze more than three times: 3 points; iii) wheezing rale: 2 points; iv) sudden attack of wheezing: 1 point; and v) other specific medical history: 1 point. The patients with the total score  $>5$  points could be diagnosed as infantile asthma. The diagnostic criteria of rickets referred to the textbook of Pediatrics (9). i) Serum 25 (OH) D 3 and 1, 25 (OH) 2D3 were significantly lower than normal. ii) Increased excretion of alkaline phosphatase in urine. iii) In the early stage of X-ray, the calcification preparation line of long bone metaphysis was blurred. In stadium acmes, calcification preparation line disappeared, the epiphyseal end widened, the metaphysis changed in the shape of cup or brush, the bone was sparse, and the shaft was curved and deformed or fractured.

**Inclusion criteria.** The inclusion criteria were: i) patients who met the above-mentioned diagnostic criteria for infantile asthma and rickets; ii) patients without other critical diseases; iii) patients who received no relevant treatment at the first visit; and iv) patients whose guardians agreed to participate in the study and complied with the doctor's advice.

#### Methods

**Collection of specimen.** Venous blood was immediately drawn from subjects in the observation group and the control group after admission. The blood was centrifuged at  $3,000 \times g$  for 15 min at  $4^{\circ}\text{C}$ , and then supernatant was collected. The expression levels of interleukin-1 (IL-1), IL-6 and IL-17 in venous blood serum were determined in accordance with instructions of the ELISA kit (cat. nos. ab46052, ab178013 and ab119535; Abcam), vitamin D and immunoglobulin E levels in venous blood serum were detected using a fully-automatic biochemical analyzer, and wheezing duration of child patients was recorded by the average time of 6 consecutive asthma attacks.

**ELISA detection.** i) Sample loading:  $100 \mu\text{l}$  standard sample or the serum to be detected was added to each well and fully mixed, and then the plate was placed for reaction at  $37^{\circ}\text{C}$  for 40 min. ii) Washing: the reaction plate was rinsed by washing liquid 4-6 times, and dried on filter paper. iii)  $50 \mu\text{l}$  distilled water and  $50 \mu\text{l}$  first antibody working fluid in the kit were added to each well (except the blank group) and fully mixed, and then the plate was placed for reaction at  $37^{\circ}\text{C}$  for 20 min. iv) Washing: The plate was rinsed by washing liquid 4-6 times, and dried on filter paper. v)  $100 \mu\text{l}$  enzyme-labeled antibody working fluid in the kit was added to each well, and then the plate was placed for reaction at  $37^{\circ}\text{C}$  for 10 min. vi) Washing: The plate was rinsed by washing liquid 4-6 times, and dried on filter paper. vii)  $100 \mu\text{l}$  substrate working fluid in the kit was added to each well, and then the plate was placed for reaction in a dark place at  $37^{\circ}\text{C}$  for 15 min. viii)  $100 \mu\text{l}$  stop buffer in the kit was added to each well and fully mixed. ix) The absorbance value was detected by a microplate reader at 450 nm.

**Detection using the fully-automatic biochemical analyzer.** The collected venous blood serum was put into the fully-automatic biochemical instrument. Vitamin D and immunoglobulin

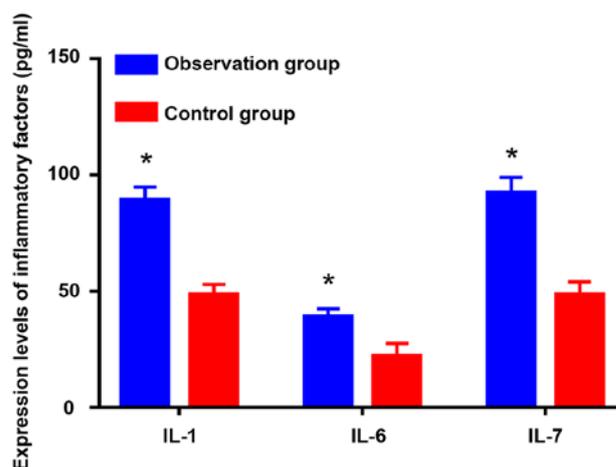


Figure 1. Expression levels of inflammatory factors by t-test. Expression levels of IL-1, IL-6 and IL-17 in serum of the observation group were significantly higher than those of the control group ( $P < 0.05$ ). Compared with that in the control group,  $*P < 0.05$ . IL, interleukin.

levels in serum were detected after the detection parameters were set.

**Statistical analysis.** SPSS 20.0 software (SPSS, Inc.) was adopted for statistical analysis in this study. Enumeration data were expressed as mean  $\pm$  standard deviation. The t-test was used for data in line with normal distribution and homogeneity of variance, and the corrected t-test was utilized for data in line with normal distribution and heterogeneity of variance. Non-parametric test was adopted for data not in line with normal distribution and homogeneity test of variance, rank sum test for rank data, and Chi-square test for enumeration data. Pearson correlation analysis was utilized for correlation analysis.  $P < 0.05$  was considered to indicate a statistically significant difference.

#### Results

**Detection of IL-1, IL-6 and IL-17 expression levels in serum using ELISA.** As shown in Fig. 1, the expression levels of IL-1 ( $47.45 \pm 4.77$  pg/ml), IL-6 ( $29.87 \pm 5.21$  pg/ml) and IL-17 ( $52.734 \pm 5.63$  pg/ml) in serum of the observation group were significantly higher than those of the control group ( $84.39 \pm 4.88$ ,  $43.45 \pm 3.89$  and  $91.33 \pm 5.63$  pg/ml) ( $P < 0.05$ ).

**Detection of vitamin D and immunoglobulin levels using fully-automatic biochemical analyzer.** As shown in Figs. 2 and 3, vitamin D ( $1.12 \pm 0.57$  mmol/l) and immunoglobulin ( $12.28 \pm 3.44$  g/l) levels in venous blood serum of the observation group were remarkably lower than those of the control group ( $2.38 \pm 0.87$  mmol/l,  $14.66 \pm 4.34$  g/l) ( $P < 0.05$ ).

**Wheezing duration.** The wheezing duration of child patients in the observation group ( $23.32 \pm 6.39$  sec) was evidently longer than that in the control group ( $14.87 \pm 5.49$  sec) ( $P < 0.05$ ; Fig. 4).

**Correlation analysis.** According to calculation, the r value of correlation analysis between IL-17, IL-1 and IL-6 expression levels and wheeze duration was 0.875, 0.851 and 0.882,

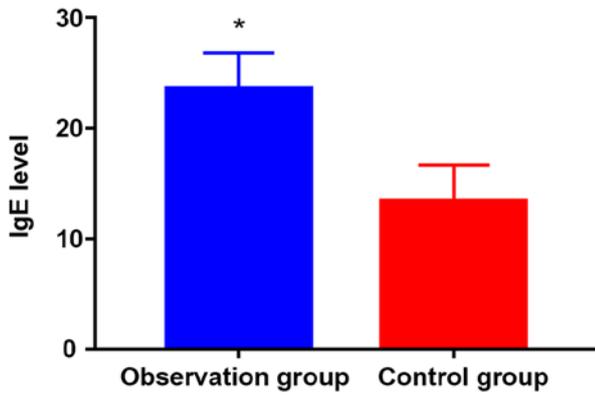


Figure 2. IgE level by t-test. IgE level in observation group were higher than those of control group ( $P < 0.05$ ). Compared with that in the control group,  $^*P < 0.05$ . IgE, immunoglobulin E.

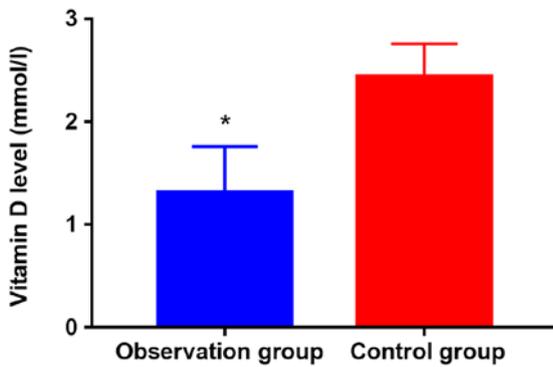


Figure 3. Vitamin D level by t-test. Vitamin D level in observation group were lower than those of control group ( $P < 0.05$ ). Compared with that in the control group,  $^*P < 0.05$ .

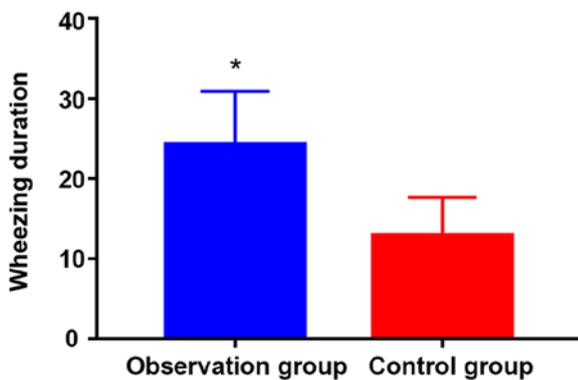


Figure 4. Wheezing duration by t-test. The wheezing duration of child patients in observation group was evidently longer than that in the control group ( $P < 0.05$ ). Compared with that in the control group,  $^*P < 0.05$ .

respectively, demonstrating that inflammatory factors are positively related to wheezing duration (Figs. 5-7). The  $r$  value of correlation analysis between vitamin D and wheezing duration was  $-0.815$ , suggesting that there is a negative correlation between them (Fig. 8). The  $r$  value of correlation analysis between immunoglobulin E level and wheezing duration was  $0.928$ , indicating that there is a negative correlation between them (Fig. 9).

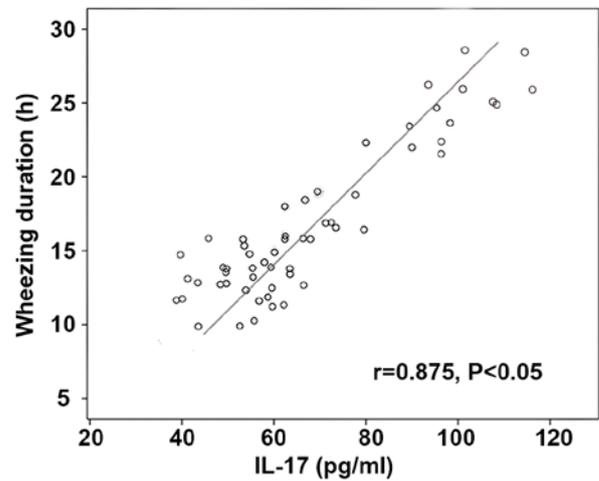


Figure 5. Correlation between wheezing duration and IL-17 by Pearson correlation analysis. IL-17 expression level is positively related to wheezing duration ( $P < 0.05$ ). IL, interleukin.

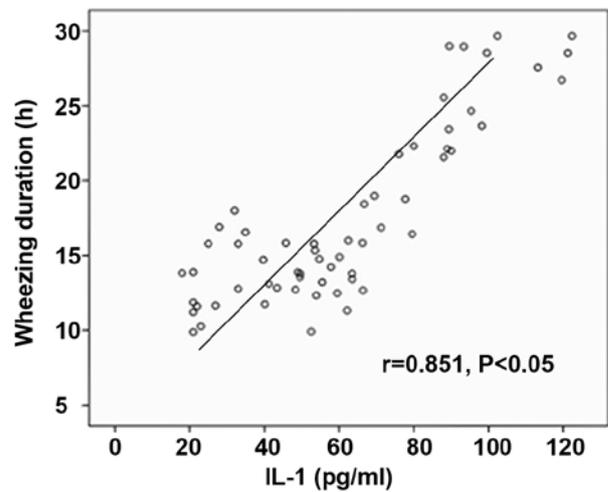


Figure 6. Correlation between wheezing duration and IL-1 by Pearson correlation analysis. IL-1 expression level is positively related to wheezing duration ( $P < 0.05$ ). IL, interleukin.

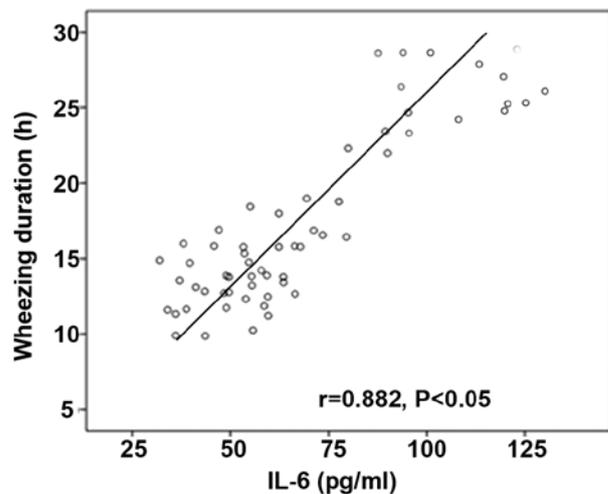


Figure 7. Correlation between wheezing duration and IL-6 by Pearson correlation analysis. IL-6 expression level is positively related to wheezing duration ( $P < 0.05$ ). IL, interleukin.

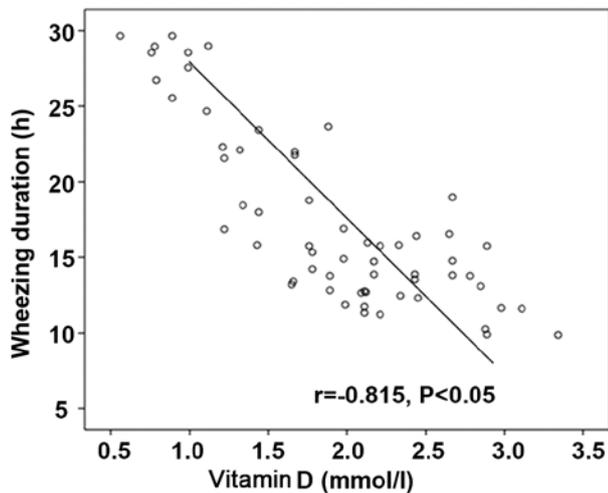


Figure 8. Correlation between wheezing duration and vitamin D by Pearson correlation analysis. Wheezing duration is negatively related to vitamin D level ( $P < 0.05$ ).

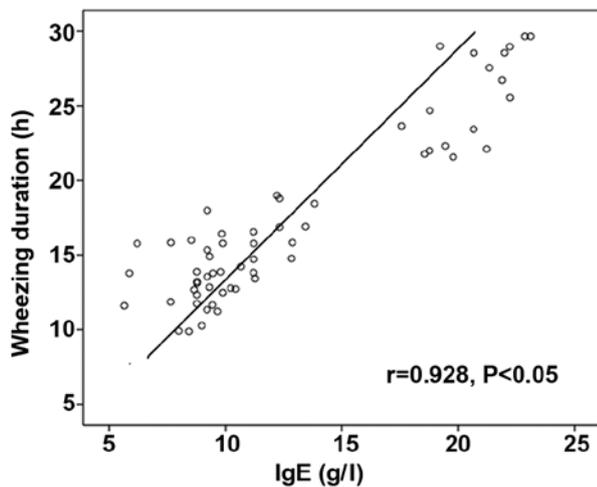


Figure 9. Correlation between wheezing duration and IgE by Pearson correlation analysis. Wheezing duration is positively related to IgE level ( $P < 0.05$ ). IgE, immunoglobulin E.

## Discussion

Rickets is a common complication of infantile asthma in clinical practice, which is characterized by frequent wheezing attacks and high severity and difficult to control. It has been found clinically that vitamin D or calcium is able to effectively relieve wheezing symptoms in asthmatic infants with rickets, suggesting the close relationship between infantile asthma and rickets. Moreover, a number of risk factors are associated with the disease (10,11). As a steroid hormone, vitamin D plays an important role not only in skeletal diseases such as rickets, but also in autoimmune diseases, cardiovascular diseases and cancer (12). Vitamin D exerts an indispensable role in immune regulation. The study indicates that the appropriate amount of vitamin D can effectively reduce the expression of T-helper (Th)-1 and Th2 cells, inhibit cellular immune-mediated inflammation, and inhibit the expression of inflammatory factors (13).

Vitamin D can also inhibit the expression and secretion of IL-4 in bronchoalveolar lavage fluid, so as to inhibit airway inflammation. Additionally, vitamin D inhibits inflammation by inhibiting IL-10 and transforming growth factor (TGF)- $\beta$  (14). Furthermore, vitamin D can promote IL-10 in patients with asthma tolerance induced by steroids (15,16). During clinical treatment, the acute attack of asthma and tolerance of asthma to glucocorticoid can be reduced by vitamin D (17-19). Given that there is a close relationship between vitamin D and rickets, vitamin D may be a risk factor for infantile asthma with rickets. Pro-inflammatory factors mainly secreted by Th17, including IL-1, IL-6 and IL-17, were responsible for immune regulation during asthma attack (20).

It has been proven that IL-1, IL-6 and IL-17 can not only aggravate acute inflammation during asthma attack, but also induce multiple immune cells to secrete inflammatory cytokine IL-6, and increase airway mucus secretion, so as to play important roles in airway remodeling (21). Therefore, IL-1, IL-6 and IL-17 are of significance during all aspects of asthma attack. Immunoglobulin E is considered to be one of the important substances that trigger the immune response of asthma, and it is a key factor in the pathogenesis of asthma. Immunoglobulin E binds to a large number of immune cells, causing the body to be in a higher state of sensitization (22,23). When the body is stimulated by allergen, inflammation can occur, the body's physiological function is disturbed, and asthma attack is induced (24,25). The study indicates that the level of immunoglobulin E in serum of asthmatic children is significantly higher than that of normal children, and the level of immunoglobulin E is closely related to the severity of asthma (23). Therefore, immunoglobulin E is also considered as one of the risk factors. The results of this study displayed that IL-1, IL-6, IL-17 and immunoglobulin E levels in serum of infantile asthma with rickets were significantly higher than those in simple asthma children, and the vitamin D level was remarkably lower than that in children with simple asthma. The results of correlation analysis proved that inflammatory factors and immunoglobulin E were positively related to infantile asthma with rickets, but the vitamin D level was negatively associated with infantile asthma with rickets. Therefore, the high expression of inflammatory factors and immunoglobulin E and low expression of vitamin D are considered as risk factors of infantile asthma with rickets.

However, asthmatic infants with low Vitamin D, but not rickets may be an interesting control to be explored.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Authors' contributions

AA was involved in writing the manuscript. AA and BY analyzed general data of patients and collected the specimens. PX performed ELISA. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) and informed consents were signed by the guardians.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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