Serum vitamin D level in mice with allergic rhinitis is correlated with inflammatory factors

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Abstract: Objective: This study aimed to explore the correlation between serum vitamin D and inflammatory factors in mice with allergic rhinitis. Methods: Female BALB/c mice in SPF grade were used to construct allergic rhinitis model by systemic injection and repeated nasal antigens. 12 allergic rhinitis mice without other treatment were treated as group A, another 12 allergic rhinitis mice treated with vitamin D₃ were selected as group B, and group C included 12 mice that received PBS injection. Nasal symptoms, behavioral scores, serum vitamin D levels, nasal mucosal pathology HE staining, serum inflammatory factors IL-4 and IFN-γ levels were compared between the groups. The relationship between serum vitamin D level and serum inflammatory factor levels were analyzed. Results: The nasal itching, sneezing, nasal secretions, behavioral scores, and total scores of group A and group B were significantly different from those of group C (P < 0.05). It showed obvious nasal mucosal edema, interrupted and lodging cilia, increased goblet cells carrying secretory bodies, and the inflammatory cells infiltrated under the mucosa in group A. They were significantly reduced in group B compared with group A. Total vitamin D levels and vitamin D₃ levels exhibited obviously difference among the groups (F = 53.19, P < 0.05). IL-4 and INF-γ levels in group A and group B were markedly higher than those in group C (P < 0.05). IL-4 decreased following serum vitamin D level elevation (Y = -3.3515X + 122.04, R² = 0.9984). Conclusion: Vitamin D in young mouse is implicated with allergic rhinitis and attenuated inflammation. Vitamin D level was significantly negatively correlated with IL-4, suggesting that vitamin D was closely related to inflammation.

Keywords: Allergic rhinitis, vitamin D, IL-4, IFN-γ

Introduction

Allergic rhinitis currently shows the highest incidence and accounts for about 50% of the allergic diseases [1]. Nasal itching, repeated sneezing, and nasal congestion are typical clinical symptoms of type I allergic rhinitis [2]. It is mainly caused by the immunological reaction through the antigen binding with the IgE antibody from mast cells in the surface of the nasal mucosa under the sensitized state [3]. The release of various chemical mediators, including histamine, leukotrienes, and platelet-activating factors, induce nasal glandular secretion and nasal vascular response, which in turn cause itch, sneezing, salivation, and nasal congestion [2]. It was shown that IL-4 level was significantly elevated in nasal biopsies and IL-4 protein expression was upregulated in nasal secretions in the early stage of allergic rhinitis [4]. IFN-γ is also one of the important inflammatory mediators, which is mainly secreted by Th-1 cells. It was found that IFN-γ content was high in non-allergic diseases, but was not observed in allergic T cell [5]. Numerous studies demonstrated that vitamin D can regulate the immune system through binding with vitamin D receptor (VDR) on the surface of various immune cells, especially T lymphocytes, which can be induced by vitamin D for proliferation and differentiation [6, 7]. Allergic rhinitis represents an immune imbalance disease. This study aimed to explore the correlation between serum vitamin D and inflammatory factors in mice with allergic rhinitis.

Materials and methods

Experimental animals

Thirty-six female BALB/c mice aged 4-6 weeks and weighted 20 ± 2 g in SPF grade were purchased from Experimental Animal Center of Huazhong University of Science and Technology.
They were randomly divided into 3 groups according to different treatment methods. All operations and protocols on animals were approved by the Laboratory Animal Ethics Committee of Ningbo Yinzhou Second Hospital.

Reagents and instruments

Ovalbumin was purchased from Sigma. B. pertussis was purchased from Japan Beili Institute. 1,25-dihydroxyvitamin D₃ was purchased from Sigma. Aluminum hydroxide adjuvant was purchased from Thermo. IL-4 and IFN-γ ELISA kits were purchased from Boster. High Performance Liquid Chromatography Tandem Mass Spectrometry was purchased from Applied Biosystems.

Treatment methods

The mouse allergic rhinitis model was established according to the reports [7, 8]. 0.2 ml antigen adjuvant containing 1 mg/ml ovalbumin, 10¹⁰/ml pertussis, and 2 mg aluminum hydroxide was injected to the forelimb and hind limb paralysis for sensitization. Sensitization was enhanced on the 5th day after initial injection. 0.5 mg of ovalbumin was dissolved in 1 ml normal saline and injected into the back of young mice. After initial sensitization for 7 days, 1 g/L ovalbumin was dropped to the nose for 7 days to establish allergic rhinitis model.

12 allergic rhinitis mice without other treatment were treated as group A, another 12 allergic rhinitis mice treated with vitamin D₃ were selected as group B, and 12 mice received PBS injection were considered as group C.

Nasal symptom and behavioral evaluation criteria

From the first stimulation, the mice were observed for nasal itching, sneezing, salivation symptoms, and behavior changes within 30 minutes after each challenge. (1) Nasal itching: score 1 for intermittently rubbing the nose several times; score 2 for constantly scratching the nose and face; score 3 for continuous scratching and rubbing the nose and face. (2) Sneezing: score 1 for 1-3 sneezes, score 2 for 4-10 sneezes, and score 3 for 10 or more sneezes. (3) Nasal secretions: The anterior nostril was considered as limit. The flow to the upper was treated as 1 point, to the bottom was treated as 2 points, and to the full-face was treated as 3 points. (4) Behavioral evaluation: 1 point for mild wheezing, 2 points for obvious wheezing, and 3 points for sputum-like wheezing.

HE staining

The fresh nasal mucosa of the mice was fixed with 4% paraformaldehyde, stained by HE, and observed by optical microscopy.

Vitamin D level detection

5 ml venous blood was collected to extract serum. The 25OHD₂ and 25OHD₃ levels in the serum were determined by high performance liquid chromatography-tandem mass spectrometry.

Inflammatory factors detection

The spleen of the mouse was taken out under aseptic conditions. The tissue was grinded with lymphocyte separation solution and filtered through a sterile 200 mesh. The spleen cells were resuspended in RPMI1640 incomplete medium, together with ethylenediaminetetraacetic acid (EDTA)-ammonium chloride (NH₄Cl). The spleen cells were collected by centrifugation at 2184 r/min for 15 min and seeded in 48-well plate at 5×10⁶/well. After cultured in a 37°C incubator for 72 hours and centrifuged at 4°C and 3500 r/min for 10 min, and the supernatant of the cells was collected. The levels of IL-4 and INF-γ in the supernatant were detected by ELISA kit according to the instructions.

Statistical analysis

All data analyses were performed on SPSS 19.0 statistical software. The measurement data were compared by One-way analysis of variance and SNK-q test. The correlation between vitamin D and inflammatory factors were analyzed by simple linear analysis. P < 0.05 was considered as statistical significance.

Methods

Nasal symptoms and behavioral scores comparison: The nasal itching, sneezing, nasal secretions, behavioral scores, and total scores of group A and group B were significantly different from those of group C (P < 0.05). Group B mice exhibited statistically different symptoms compared with group A, including nasal itching,
Vitamin D and inflammation of allergic rhinitis

Vitamin D level: The total vitamin D level in group C was 31.93 ± 4.28 ng/ml, of which vitamin D$_2$ level was 3.83 ± 2.17 ng/ml and vitamin D$_3$ level was 30.48 ± 4.96 ng/ml. Group A exhibited total vitamin D level at 23.79 ± 2.16 ng/ml, including vitamin D$_2$ level at 3.62 ± 1.13 ng/ml and vitamin D$_3$ level at 20.38 ± 3.04 ng/ml. Group B demonstrated total vitamin D level at 29.16 ± 4.37 ng/ml, containing vitamin D$_2$ level at 3.69 ± 1.73 ng/ml and vitamin D$_3$ level at 26.75 ± 5.13 ng/ml. Total vitamin D levels and vitamin D$_3$ levels exhibited significant difference among the groups (F = 53.19, P < 0.05) (Figure 2).

HE staining: Our result indicated obvious nasal mucosal edema, interrupted and lodging cilia, increased goblet cells carrying secretory bodies, and the inflammatory cells infiltrated under the mucosa in group A. They were significantly alleviated in group B compared with group A (P < 0.05). The integrity of nasal mucosa was found in group C (Figure 1).

Inflammatory factor levels: IL-4 and INF-γ levels in group A and group B were markedly higher than those in group C (P < 0.05). IL-4 level in group A was significantly higher than group B (P < 0.05). IFN-γ level in group A was statistically lower than that of group B (P < 0.05) (Table 2).

Correlation analysis between vitamin D level and inflammatory factor levels: It was revealed that IL-4 was decreased following serum vitamin D level elevation (Y = -3.3515X+122.04, R$^2$

Table 1. Nasal symptoms and behavioral scores comparison (X ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Nasal itching</th>
<th>Sneeze</th>
<th>Nasal secretion</th>
<th>Behavior</th>
<th>Total score</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>12</td>
<td>0.7 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>2.9 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>F</td>
<td>/</td>
<td>124.78</td>
<td>15.79</td>
<td>98.13</td>
<td>52.01</td>
<td>401.56</td>
</tr>
<tr>
<td>P</td>
<td>/</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Figure 1. Nasal mucosal HE staining. A. Group A. B. Group B. C. Group C. Magnification, 200×.

Figure 2. Vitamin D level comparison.

nasal secretions, behavioral scores, and total scores (P < 0.05) (Table 1).

HE staining: Our result indicated obvious nasal mucosal edema, interrupted and lodging cilia, increased goblet cells carrying secretory bodies, and the inflammatory cells infiltrated under the mucosa in group A. They were significantly alleviated in group B compared with group A (P < 0.05). The integrity of nasal mucosa was found in group C (Figure 1).
Vitamin D and inflammation of allergic rhinitis

Previous studies have demonstrated that among allergic diseases, the ones caused by pollen account for more than half of the patients. The reason is that pollen contains a variety of highly sensitizing proteins [17, 18]. In this study, a mouse model of allergic rhinitis was successfully established using one of the highly sensitizing proteins, ovalbumin, to test relevant serological biomarkers.

Vitamin D can be used in a variety of autoimmune diseases through regulating the immune system [19-21], such as asthma, chronic lymphocytic thyroiditis, and type I diabetes. In addition, allergic rhinitis has similar pathogenesis with asthma. Previous studies revealed that serum vitamin D (mainly on vitamin D₃) level in children with allergic rhinitis were generally low. In this study, we have found that the serum total vitamin D level and vitamin D₃ levels were significantly different among the three groups. It was observed that the inflammatory response plays an important role in the occurrence and development of allergic rhinitis. Various pro-inflammatory factors increased in allergic rhinitis patients, which promotes disease progression [22, 23]. IL-4 is a specific inducer of IgE synthesis that can promote lymphocyte proliferation, induce adhesion molecules, and facilitate IgE-mediated immune response [24]. Abnormal IFN-γ expression may represent an abnormality of inflammatory factors secretion of Th2, which can promote B cells proliferation and differentiation, induce IgE synthesis, and accelerate respiratory tract remodeling [25]. HE staining revealed that the degree of inflammation was most serious in group A, followed by group B, and no inflammatory reaction was observed in group C, further confirming the role of vitamin D in promoting inflammation. Meanwhile, our results indicated that Vitamin D₃ supplementation is of great significance for relieving the clinical symptoms and inflammatory state of allergic rhinitis. Correlation analysis demonstrated that vitamin

Table 2. Inflammatory factor levels comparison (X ± s, ng/ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>IL-4</th>
<th>INF-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>12</td>
<td>12.3 ± 0.8</td>
<td>78.4 ± 8.5</td>
</tr>
<tr>
<td>A</td>
<td>12</td>
<td>42.7 ± 2.9</td>
<td>119.8 ± 11.2</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>23.6 ± 1.4</td>
<td>132.7 ± 12.4</td>
</tr>
<tr>
<td>F</td>
<td>/</td>
<td>89.78</td>
<td>95.41</td>
</tr>
<tr>
<td>P</td>
<td>/</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Discussion

Allergic rhinitis is one of the common pediatric diseases with high incidence worldwide [9-11]. In recent years, with intensified air pollution and environmental deterioration, the incidence of allergic rhinitis and some respiratory diseases have increased significantly, especially in developed cities with higher industrialization and living standards. In China, the poor quality of the atmosphere due to urban construction and resource exploitation may worsen the situation. Moreover, the underbalanced and irregular diet is also an important cause of allergic rhinitis. Allergic rhinitis belongs to the type I allergic reaction that occurs in the nasal mucosa. The most important pathological features are telangiectasia and increased glandular secretion [12-14]. Clinically, the main manifestations include sneezing, nasal discharge, nasal congestion, nasal itching, etc., which affects the normal life and even induces complications, such as asthma and sinusitis [15, 16].
Vitamin D level was significantly negatively correlated with IL-4, which may be caused by the vitamin D deficiency leads to immunity decrease, and inflammatory state aggravation, thereby stimulating the synthesis and expression of IL-4.

Conclusion

Vitamin D in young mouse is implicated to allergic rhinitis and it alleviates the inflammatory response. Vitamin D level was significantly negatively correlated with IL-4, suggesting that vitamin D was closely related to inflammation.

Disclosure of conflict of interest

None.

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References

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