Serum level and clinical significance of vitamin E in children with allergic rhinitis

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Abstract
Background: Allergic rhinitis (AR) has an increasing prevalence in children and its etiology has aroused wide concern. This study aimed to investigate the association between serum concentrations of vitamin E and allergic rhinitis (AR) to determine if the vitamin E level is correlated with the occurrence and severity of AR.

Methods: A total of 113 children were enrolled in this cross-sectional study. Sixty-five children in the outpatient group were diagnosed with AR, and 48 healthy children were recruited as controls. All subjects underwent serum vitamin E measurements. Serum total IgE (tIgE), the five most common allergen-specific IgE (sIgE) levels and skin prick test (SPT) were measured in children with AR. The severity of AR was assessed with the nasal symptoms score.

Results: Serum vitamin E levels were significantly lower in the AR group than in the normal children (p<0.001). A significant negative correlation was observed between serum vitamin E levels and sIgE as well as the SPT grade. Serum vitamin E levels were also inversely related to the nasal symptoms score; however, statistical significance was not found.

Conclusions: A significantly lower vitamin E level was found in children with AR. Lower serum vitamin E levels play a role in the occurrence of AR in children. However, serum vitamin E levels were not statistically correlated with the severity of AR.

Background
Allergic rhinitis (AR) is widely defined as nasal mucosal inflammation and mostly reflects IgE-mediated type 1 hypersensitivity to common environmental and food antigens. Typical symptoms of the disease are nasal congestion, itching, sneezing and rhinorrhoea [1]. In patients with AR, elevated total IgE (tIgE) or specific IgE (sIgE) antibody levels are commonly observed. Thus, the serum levels of tIgE and sIgE after exposure to common allergens be used as a universal indicator for the diagnosis of AR and its severity [2]. In recent years, the prevalence of AR and asthma is increasing worldwide, especially in children. The mechanism has not been fully elucidated hitherto, and increase in ambient PM$_{2.5}$ levels and antioxidants vitamin D deficiency may be the main risks factors [3–5], while the association with antioxidants vitamin E deficiency is still unclear.
Vitamin E, a essential nutrient for reproduction, is synthesized in plant organisms and composed of eight fat-soluble compounds (α-, β-, γ-, δ-tocopherol, and α-, β-, γ-, δ-tocotrienol) [6]. Some studies have confirmed that that oxidative stress plays an important role in the pathogenesis of several allergic diseases, including AR [7]. Vitamin E is a peroxyl radical scavenger that can protect neurons and respiratory mucosa from oxidant damage, while vitamin E can significantly reduce the incidence of asthma and relieve respiratory mucosa inflammation [8, 9].

The relationship between dietary antioxidants and allergic diseases has been previously reported. Vitamin E intake can protect against the development of atopy and wheezing in young children [10]. However, vitamin E supplementation did not reduce the severity of AR or the duration that allergy medications were used to control symptoms in adults [11].

To date, there have been few researches on the association between AR and serum vitamin E in children. Many studies have focused on allergic diseases such as asthma, wheezing, and atopic dermatitis. Given that the potential therapeutic value of vitamin E in other allergic diseases, we addressed this deficiency by concentrating on the association between vitamin E and AR in children and determining the correlations between vitamin E levels and sIgE/tIgE, SPT grade and nasal symptoms score. The aim of this work was to evaluate the possible association between vitamin E levels and the occurrence and severity of AR in children.

Methods
Research Subjects
A total of 65 children aged from 6 to 14 years who were diagnosed with AR at the outpatient department of Anhui Provincial Hospital, Anhui, China, between September 2017 and September 2018 were included in the study. Forty-eight matched healthy children were recruited as controls at the same time. All studies are approved by Anhui Provincial Hospital Ethics Committee. The parents' informed consent was obtained at enrolment. The diagnosis of AR was defined according to Allergic Rhinitis and Its Impact on Asthma guidelines [1]. The inclusion criteria were 2 or more symptoms of nasal congestion, itching, sneezing and rhinorrhhea, totalling more than one hour a day; definite provocative factors; and skin prick test results positive for at least one allergen. All patients had
symptoms without evidence of infection, sinusitis, otitis media, nasal polyps, nasal septum deviation, atopic dermatitis, lung diseases, systemic diseases and anatomical abnormalities. Within 3 months of the study, none used vitamin supplements, corticosteroids (nasal or systemic), anti-inflammatory drugs, anti-leukotrienes, cromolyn, or immunotherapy.

**Blood samples**

Venous blood samples (2 ml) were obtained from children who had fasted for 8 hours; the samples were then left to clot for 60 minutes at room temperature. After centrifugation for 15 minutes at 1500 rpm, serum samples were stored and frozen at −80 °C until the day of the assay.

Serum levels of vitamin E were determined by HPLC (LC-20AD; Shimadzu, Japan). All measurements were performed by the same researcher in the same laboratory and using the same kit.

Serum slgE and tlgE were measured using ImmunoCAP (Phadia AB, Uppsala, Sweden) [12]. Specific IgE concentrations were measured for the five most common allergens found in southern China (Dermatophagoides farina, cockroach, dandelion, ragweed, birch). The slgE value of 0.35 kU/L or above (0.35-100 kU/L) was considered positive. slgE were classified into 6 degrees [13]: grade 0: values < 0.35 kU/L, 1 grade: 0.35 ≤ values ≤ 0.69 kU/L, 2 grade: 0.7 ≤ values ≤ 3.4 kU/L, 3 grade: 3.5 ≤ values ≤ 17.4 kU/L, 4 grade: 17.5 ≤ values ≤ 49.9 kU/L, 5 grade: 50 ≤ values ≤ 100 kU/L, and 6 grade: values ≥ 100 kU/L.

**Symptoms Score**

Children were instructed to complete the nasal symptom score questionnaire (maximum 18). We scored the severity and duration of three nasal symptoms (nasal congestion, rhinorrhea and sneezing). The severity and duration of each nasal symptom were scored on a 3-point scale as follows: not at all/none of the time was 0; mild, well-tolerated/once in a while was 1; moderate, somewhat bothered/sometime was 2; and severe, very bothered/most or all time was 3.

**Skin prick testing**

SPT was performed using standard allergen extracts (ALK Horsholm, Denmark) of 8 inhalant allergens (Dermatophagoides farina, Dermatophagoides pteronyssinus, Blomia tropicalis, birch, mugwort, ragweed, dandelion, cat hair, and cockroach). Histamine dihydrochloride (10 mg/ml) and physiological sodium chloride (9 mg/ml) served as positive and negative controls, respectively. SPT was performed
on the volar surface of the forearm with a single peak lancet, and wheal sizes were measured after 15 minutes. The skin index (SI) was calculated as SI = allergen diameter/histamine diameter; values were recorded as normal: 0, negative; level one: "+", SI < 0.5; two: "++", 0.5 < SI < 1; three: "+++", 1.0 ≤ SI < 2.0; four: "++++", SI ≥ 2.0. If the reaction to the physiological sodium chloride prick was positive, skin irritation should be considered and eliminated. Those with a negative histamine reaction were also eliminated, and the grade one and above level of the pricking test were recorded as positive. The positive skin prick test response to Dermatophagoides farina (Df) was included in the correlation study.

Statistical analysis
All statistical analyses were conducted using SPSS22.0 (SPSS, USA). Continuous variables were expressed as the mean ± standard deviation (SD) (± S); differences between groups were determined by Student’s t-test or analysis of variance for continuous variables and by the Pearson chi-square test for categorical variables. Multiple logistic regressions were designed to adjust the simultaneous effects of confounding variables such as age, sex and body mass index on the vitamin E level between the two groups. Correlations between serum vitamin E and sIgE, total IgE, the nasal symptoms score and SPT grade were calculated with Spearman's correlation test. A value of p < 0.05 was considered significant.

Results
Comparison of general characteristics and serum VE levels between AR children and control
Sixty-five children who were eventually diagnosed with AR were enrolled in the study, and 48 healthy children were recruited as a control group in the same period. There were no significant differences regarding age, sex or body mass index between the two groups. The preliminary results revealed that serum vitamin E levels (ng/ml ± SD) were significantly lower in AR children (6.61 ± 1.37) than in normal children (9.21 ± 1.69; p < 0.001). Serum tIgE (IU/ml) was significantly higher in AR children (289.0 ± 101.1) than in normal children (82.5 ± 18.9; p < 0.001). Data are shown in Table 1. After adjusting the model for sex, age and body mass index, the analysis revealed that serum vitamin E (odds ratio [OR], 0.263; 95% confidence interval [CI], 0.161–0.429; p < 0.001) contributed significantly
Correlative analysis of serum VE level and relevant indicators of AR

In the children with AR, there was a significant inverse correlation between serum vitamin E levels and sIgE (r = -0.608; p < 0.001) (Fig. 1A). However, there was no significant inverse correlation between serum vitamin E and tIgE (r = -0.178; p = 0.156) (Fig. 1B), and there was no significant inverse correlation between serum vitamin E levels and the nasal symptoms score (r = -0.216; p = 0.084) (Fig. 1C). The results of the skin prick test showed that 62 children were allergic to dermatophagoids, of whom 58 were allergic to *D. farina*, which was the most common allergen. There was a significant inverse correlation between serum vitamin E levels and Df SPT grade (r = -0.550; p < 0.001) (Fig. 1D).

Discussion

We investigated the association between serum vitamin E and AR in children aged 6-14 years. The levels of vitamin E in children with AR were lower than normal children, and an association was found between vitamin E levels and AR. The results remained significant even after adjusting for confounding factors related to vitamin E.

The relationship between AR prevalence and serum vitamin E levels is controversial in existing studies. In several cohort studies, it was found that maternal vitamin E intake from food during pregnancy was inversely related to the risk of AR in children [14, 15]. Similarly, high-dose vitamin E supplementation in combination with routine treatment may be valuable to improving symptoms in patients with seasonal allergic rhinitis [11]. In contrast, some reports found no association between AR and vitamin E intake [16, 17]. The differences may derive from the following reasons: 1) Children
may need more supplementation in the vitamin E due to their faster metabolic rate 2) the
discrepancy in dietary structure and nutritional status of different regions.

Obtaining a thorough history and physical examination as well as identifying specific allergic triggers
are required to establish the clinical diagnosis of allergic rhinitis. Allergen-specific IgE tests and skin
prick tests are the main methods for determining allergens. Each has its advantages and cannot be
replaced by the other. Therefore, we analyzed the correlation between the two results and serum
vitamin E levels of children with AR, attempting to link vitamin E with the diagnosis of AR. In this
study, 62 children with AR were found to be allergic to dermatophagoids. *D. farinae*, the most
common allergen in children with AR, followed by cockroach and birch. At the same time, our results
showed a significant inverse correlation between serum vitamin E levels and Df SPT grade.

McCann W A et al. [18] demonstrated the diagnostic value of serum sIgE in allergic diseases, with the
sensitivity fluctuating between 84% and 95% and the specificity fluctuating between 85% and 94%.
Given that serum immunoglobulin E (IgE) can be synthesized even before clinical symptoms occur,
and elevated IgE levels are one of the indicators of type I allergic reactions, we measured the serum
tIgE and sIgE levels and found that sIgE was negatively correlated with vitamin E. Correspondingly,
Fogarty et al. [19] revealed that higher-dose of vitamin E intake were associated with lower serum IgE
concentrations and a lower frequency of allergic sensitization. Because of the diagnostic value of SPT
and sIgE in AR, we investigated the association between SPT and sIgE and serum vitamin E and found
a statistically significant inverse correlation, which may reflect the inverse correlation between serum
vitamin E level and AR. However, the correlation between tIgE and vitamin E was not statistically
significant. This may be because many factors can lead to increasing tIgE levels in the body, and
approximately one-third of patients with AR were in the normal range.

Vitamin E, a fat-soluble vitamin, is one of the most important antioxidants and is closely correlated
with immune function [20]. At the same time, the production of reactive oxygen species (ROS) and
oxidative stress are related to allergic inflammation. Vitamin E metabolites have been identified as
potential regulator associated with immune, inflammatory responses, lipid metabolism, neuronal cell
protection and vessel homeostasis in vivo [21, 22]. Although vitamin E has a positive antioxidant
effect and oxidative stress has a role in the development of AR, the association between vitamin E and AR has not been proven beyond any reasonable doubt. Our data provide strong evidence for the link between vitamin E and allergic rhinitis. T helper 2 (Th2) cytokines interleukin-4 (IL-4), IL-5, IL-9 and IL-13 play key roles in AR propagation and in the maintenance of allergic inflammation, while T helper 1 (Th1) cytokines play the opposite role. In vitro, vitamin E promotes the secretion of Th1 cytokine and inhibits Th2 cytokines secretion [23, 24]. Vitamin E also reduces the secretion of IL-4 in human peripheral blood T cells [25]. IL-4 can promote the production of IgE antibodies by B-cells and is one of the key cytokines in the development of allergic inflammation. In addition, vitamin E inhibits the activity of cyclooxygenase, indirectly inhibits the synthesis of arachidonic acid-derived prostaglandin E2 (PGE2), and PGE2 has been involved in shifting the balance of Th1/Th2 cells and their cytokines towards a Th2 profile [26, 27]. In conclusion, these findings support that vitamin E might be protective against allergic sensitization.

Our study showed no significant inverse correlation between serum vitamin E levels and nasal symptoms scores. In a controlled study, 112 patients with seasonal AR had lower nasal symptoms scores after high-dose vitamin E supplementation (800 IU/d) during the hay fever season [11]. In contrast, another study showed that no significant effect on nasal symptoms in 63 patients with perennial AR after normal-dose vitamin E supplementation (400 IU/d) [17]. The differences may be due to the subjectivity of the symptoms score and the fact that it is targeted at children; a larger sample and further clinical studies are needed to evaluate this relationship.

Conclusions
Our study is one of a few to investigate the relationship between serum vitamin E and AR in children, and it is the first to explore the potential association of IgE and SPT. Our study has multiple strengths, including complete data on both serum vitamin E, IgE and SPT results of children with AR. Serum vitamin E, IgE and SPT results were also measured by trained health professionals.

We recognize that there are some limitations in this study. First, as with other clinical case-control trials, the present study is prone to recall, selection, and measurement bias. Second, it is possibility that some unknown confounding factors were not adjusted for in this study; this problem exists in any
observational design. Third, we utilized sIgE measurements from a single allergen, which may lead to the problem of partial generalization. Fourth, due to the cross-sectional design of this study, it was impossible to infer causality.

In view of the significantly serum lower vitamin E levels in children with AR, we speculated that lower serum vitamin E levels play a role in the occurrence of AR in children, providing a theoretical basis for designing effective intervention programs for children with AR. However, serum vitamin E levels were not statistically correlated with the severity of AR, requiring us to collect more samples in the future to reveal the cause.

**Abbreviations**
AR: Allergic rhinitis; VE, vitamin E; IgE: immunoglobulin E; tIgE: total IgE; sIgE: specific IgE; SPT: skin prick test; Df: Dermatophagoides farina; Th1: T helper 1; Th2: T helper 1; IL-4: interleukin-4; PGE$_2$: prostaglandin E$_2$; yr: year; N: normal; BMI: body mass index.

**Declarations**

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**Authors’ contributions**
All authors (SW, YW, CP and JS) designed the study, participated in the analytic process, critically reviewed the manuscript and approved the manuscript as submitted.

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**Availability of data and materials**
Not applicable.

**Ethics approval and consent to participate**
We conducted this study in accordance with the Declaration of Helsinki, and all participants and their parents were aware of the study protocol and signed the written consent forms. The ethical approval for the study was obtained from Anhui provincial hospital ethics committee (Certificate No. 2019-KY-09).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Figures

Correlation between serum vitamin E levels and sIgE (r=-0.608; p < 0.001) (A); correlation between serum vitamin E levels and tIgE (r=-0.178; p =0.156) (B); correlation between serum vitamin E levels and nasal symptoms scores (r=-0.216; p =0.084) (C); correlation between serum vitamin E levels and Df SPT grade (r=-0.550; p < 0.001) (D).
