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Plasma vitamin D and serum total immunoglobulin E levels in patients with seasonal allergic conjunctivitis

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ABSTRACT.

Purpose: **To evaluate plasma 25-hydroxyvitamin D and serum total immunoglobulin E (IgE) levels in patients with seasonal allergic conjunctivitis (SAC).**

Methods: **This observational case–control study involved 49 patients with SAC without any other ocular and systemic diseases, and 44 consecutive, age- and sex-matched healthy subjects. Plasma 25-hydroxyvitamin D and serum total IgE levels of all subjects were quantified with electrochemiluminescence technique. Results were compared between the groups, and p values of <0.05 were considered as statistically significant.**

Results: **No significant differences were found between the groups with respect to age (p = 0.41) and sex (p = 0.98). Plasma vitamin D levels of the subjects with SAC (median 8.03 ng/ml, range 3.00–17.97 ng/ml) were significantly lower than the control group (median 10.52 ng/ml, range 3.30–25.92 ng/ml) (p = 0.007). Serum total IgE levels of patients with SAC (median 48.65 IU/ml, range 1.77–812.00 IU/ml) were significantly higher when compared to the control group (median 32.49 IU/ml, range 0.14–104.60 IU/ml) (p = 0.003).**

Conclusions: **We found lower plasma vitamin D levels and higher serum total IgE levels in patients with SAC.**

Key words: allergy – immunoglobulin E – seasonal allergic conjunctivitis – vitamin D

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Introduction

Seasonal allergic conjunctivitis (SAC) is a common ocular surface inflammatory disease which significantly reduces the quality of life (Bielory 2006). The prevalence of allergic conjunctivitis has been estimated to be approximately 15–20% worldwide, or even higher according to more recent studies. Allergic conjunctivitis is also frequently associated with other allergic diseases, especially allergic rhinitis (Rosario & Bielory 2011).

Seasonal allergic conjunctivitis is a type 1 hypersensitivity reaction mediated by immunoglobulin E (IgE), which is elicited by airborne environmental antigens (usually pollens) (Ono & Abelson 2005). When an allergen enters the ocular surface, it is processed by antigen-presenting cells at first and presented to T helper lymphocytes as a peptide fragment, directing them to T helper type 2 (Th_2) cells. These cells produce interleukins and other specific cytokines stimulating the B-cell production of IgE, which then binds to the surface of mast cells. When specific allergens reach to the ocular surface, they react with specific IgE antibodies bound to sensitized mast cells leading them to degranulate and release a considerable number of preformed and newly formed mediators. Histamine, which is the predominant mediator, together with other mediators and chemotactic factors induces the major clinical signs and symptoms of allergic conjunctivitis (itching, conjunctival hyperaemia, tearing, chemosis and lid oedema) (Ono & Abelson 2005).

Allergic diseases, which were very rare a century ago, are frequently encountered recently and affect as much as 40% of the population in developed countries (Pawankar et al. 2011). This rise in the incidence of allergic diseases is attributed to several factors such as industrialization, lifestyle changes, dietary intake and exposure to environmental factors such as air pollution (Prescott 2013). Also more recently, especially upon the discovery of the regulatory effects of vitamin D on the immune system (Adams & Hewison 2008), variations in vitamin D status have been implicated in the development of allergic diseases (Frieri & Valluri 2011). It has been suggested that vitamin D has a significant role, mainly immunomodulatory, in human physiology beyond skeletal health and calcium homeostasis (Adams & Hewison 2008). Altered vitamin D status has been linked to a diverse group of diseases such as allergies (Frieri & Valluri 2011; Allen et al. 2013), cancers (Feskanich et al. 2004), autoimmune (Munger et al. 2006) and

infectious diseases (Cannell et al. 2006). Of these, allergic diseases are of particular interest and association of vitamin D with various allergies such as asthma, food allergy and allergic rhinitis has been proposed (Frieri & Valluri 2011; Litonjua 2012; Allen et al. 2013).

Although the most effective means of managing allergic diseases is to identify and avoid exposure to the offending antigen, this is usually not possible for allergic conjunctivitis as most antigens are airborne (Bielory 2008). Pharmacological treatment of SAC is the mainstay, but it may still have some limitations such as non-compliance to the therapy and ineffectiveness in preventing the relapses. So, understanding the influence of environmental factors to the pathogenesis of allergic diseases, that is, vitamin D deficiency, could modify our dealing of these diseases.

Therefore, we aimed to investigate plasma vitamin D levels of patients with SAC and compare with healthy controls without any allergies. We are unaware of any previous reports demonstrating plasma vitamin D levels in patients with seasonal allergic conjunctivitis.

Methods

Study population

This prospective, single-centre, observational, case–control study comprised 93 subjects (49 patients with SAC and 44 control subjects). All patients were recruited in the study in May 2013, during the peak spring allergy season in Turkey (Yaylali et al. 2003), and evaluated at the Mevlana University eye clinic. Patients with a history of SAC of ≥2 years' duration, with moderate or severe signs and symptoms of clinically active allergic conjunctivitis (conjunctival hyperaemia, itching, tearing, chemosis or lid oedema) and a positive skin prick test to at least one antigen common in the geographical area were included in the SAC group. Control group was composed of 44 consecutive subjects of similar age (aged 12–44 years) and gender with normal ocular examination except refractive disorders, without any history of allergic disorders, systemic diseases or drug consumption and with negative skin prick test results. To minimize cultural influences or geographical effects on the findings, the study was conducted in the central region of Turkey, and all participants lived in the same region. Ethics committee approval (KS 2013/153) was obtained before the initiation of the study. The study was performed in compliance with good clinical practice guidelines and in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from each patient prior to enrolment in the study.

Pregnant or breastfeeding women were not included in the study. Subjects with an active ocular infection or with ocular pathology other than allergy, such as dry eye syndrome, blepharitis, uveitis and keratoconus, were excluded. Subjects who are smokers and who have a known systemic disease or history of consumption of any drugs, vitamin supplements or alcohol during the 4 weeks prior to the study were also excluded. Body mass index of all adult subjects was below 30 kg/m^2. None of the children was obese, as defined by weight under the 95th percentile of age and gender-specific centile curves.

Biochemical analysis

Fasting venous blood sample was obtained from each subject from the antecubiteal vein into EDTA anticoagulated tubes and serum separator tubes. After centrifugation at 2000 ***g*** for 15 min, serum and plasma samples were aliquoted, labelled and stored at −80°C for a maximum of 30 days until analysis. When we reached the targeted sample size, serum and plasma samples were thawed and plasma 25-hydroxyvitamin D and serum IgE levels were quantified with electrochemiluminescence technique on Elecsys 2010 analyzer (Roche Diagnostics, Mannheim, Germany). Detection limit for 25-hydroxyvitamin D was 3 ng/ml (7.5 nM). Within-run and between-run precision values determined with analysis of serum pools were as follows: 7.5% and 13.6% for low and 3.0% and 5.5% for high concentrations of 25-hydroxyvitamin D, respectively. Detection limit for IgE was 0.10 IU/ml (0.24 ng/ml). Within-run and between-run precision values determined with analysis of serum pools were as follows: 4.1% and 5.1% for low and 2.4% and 3.8% for high concentrations of IgE, respectively.

Statistical analysis

Statistical analyses were performed using a statistical program (SPSS Science, Chicago, IL, USA). The distribution of the numeric data was tested with Kolmogorov–Smirnov. Mann–Whitney *U*-test was used for testing the difference between vitamin D and IgE levels for patient and control groups. In the SAC group, vitamin D and IgE levels were compared using Pearson's correlation analysis. Mean age of groups was compared with independent-samples *t*-test, and distribution of sex between groups was compared using chi-square test with continuity correction. p values <0.05 were considered as statistically significant.

Results

Forty-nine patients with SAC (19 men and 30 women) and 44 control subjects (16 men and 28 women) were enrolled in our study. The mean ages of patients with SAC and control subjects were 25.73 ± 8.79 years (range 12–43 years) and 27.23 ± 8.61 years (range 12–44 years), respectively. No significant differences were found between the groups with respect to age ($p = 0.41$, independent-samples *t*-test) and sex ($p = 0.98$, chi-square test).

Plasma vitamin D levels of the subjects with SAC (8.19 ± 4.34 ng/ml, median 8.03 ng/ml, range 3.0–17.97 ng/ml) were significantly lower than the control group (11.66 ± 5.93, median 10.52, range 3.30–25.92 ng/ml) ($p = 0.007$, Mann–Whitney *U*-test) (Fig. 1). Serum total IgE levels of patients with SAC (159.81 ± 202.23 IU/ml, median 48.65 IU/ml, range 1.77–812.00 IU/ml) were significantly higher when compared to the control group (35.59 ± 27.42, median 32.49, range 0.14–104.60 IU/ml) ($p = 0.003$, Mann–Whitney *U*-test) (Fig. 2). There was no correlation between vitamin D and IgE levels of patients with SAC ($r = 0.15$, $p = 0.312$, Pearson's correlation analysis).

Discussion

This is a novel study evaluating the relationship between seasonal allergic conjunctivitis and vitamin D status. Plasma levels of vitamin D in patients with SAC were significantly lower than the control group ($p = 0.007$). This finding adds to the growing evidence

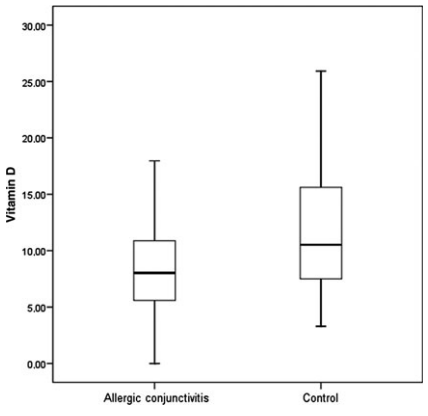

Fig. 1. Comparison of plasma 25-hydroxyvitamin D (ng/ml) levels of patients with seasonal allergic conjunctivitis and control subjects. Plasma vitamin D levels of the subjects with seasonal allergic conjunctivitis were significantly lower than the control group ($p = 0.007$, Mann–Whitney *U*-test). The central box covers the interquartile range, and the horizontal line indicates the median. The end of whiskers corresponds to the most extreme observations.

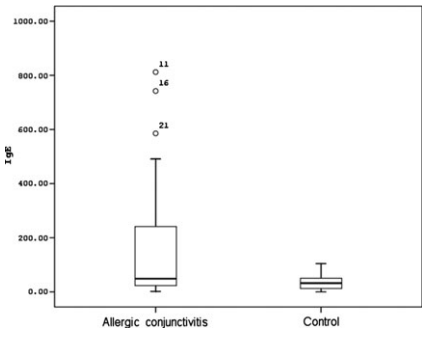

Fig. 2. Comparison of serum total immunoglobulin E (IU/ml) levels of patients with seasonal allergic conjunctivitis and control subjects. Serum total IgE levels of patients with SAC were significantly higher when compared to the control group ($p = 0.003$, Mann–Whitney *U*-test). The central box covers the interquartile range, and the horizontal line indicates the median. The end of whiskers corresponds to the most extreme observations and O indicates outliers.

of the role of vitamin D in allergic diseases.

Regulation of calcium homeostasis and skeletal health is the classic, but not the sole, function of vitamin D. After identification of vitamin D receptors in peripheral blood mononuclear cells (Provvedini et al. 1983), association between vitamin D status and immune system-mediated diseases has been implicated (Adams & Hewison 2008). Since then, a considerable number of studies had been conducted linking vitamin D deficiency with autoimmune diseases and allergic disorders (Munger et al. 2006; Frieri & Valluri 2011; Allen et al. 2013).

Helper T (Th) cells regulate the antigen-specific immune responses. Differentiation of naïve helper T cells into effector T cells (Th_1 or Th_2) or regulatory T cells is dependent on the microenvironment to which they are exposed. Th_1 cells regulate cell-mediated immune responses, whereas Th_2 cell activation is essential for antibody-mediated immunity (Hewison 2012). There should be a balance between these two subtypes of helper T cells for a normal immune response. 1,25-Dihydroxyvitamin D_3, the active form of vitamin D, has direct effects on activated helper T cells (both Th_1 and Th_2) (Adams & Hewison 2008). There are contradictory reports, of both enhancement and inhibition, about the effects of vitamin D on Th_2 cells. Also there is evidence that vitamin D can influence the function of other immunocytes such as B cells and regulatory T cells, which may explain the epidemiological association between vitamin D deficiency with both autoimmune and allergic diseases (Litonjua & Weiss 2007; Hewison 2012). Supportively, we found an association between low vitamin D levels and SAC in this present study.

The primary source of vitamin D is natural production in the skin secondary to sun exposure, as it does not naturally occur in most foods (Lamberg-Allardt 2006). It is defined that allergic diseases are more prevalent in areas away from equatorial region, and some authors pointed out that the reason may be deficiency of vitamin D (Mullins & Camargo 2012). But there is a wide spread deficiency of vitamin D reported worldwide even in sun-replete areas of the world (Binkley et al. 2007). In our study, vitamin D levels were low both in patients with SAC and in control subjects. This is in accordance with other studies from our country reporting a high frequency of vitamin D deficiency and/or insufficiency in healthy Turkish subjects (Andıran et al. 2012). Although both groups had low vitamin D levels in our study, patients with SAC had significantly lower plasma vitamin D values.

In fact, there are a considerable number of studies in the literature linking vitamin D deficiency with various types of allergic diseases. In a large survey conducted in USA, vitamin D deficiency was found to be positively correlated with prevalence of allergies (Frieri & Valluri 2011). Also there are several case–control studies showing higher rates of vitamin D deficiency among allergic patients than in controls (Ozkara et al. 2012; Allen et al. 2013). In a case–control study, vitamin D was found to be effective on Th_1/Th_2 balance in patients with allergic rhinitis and the relation between vitamin D deficiency and allergy was significant (Ozkara et al. 2012). Although most studies in the literature demonstrate a positive relationship between vitamin D deficiency and allergies, there are also some contradictory reports. Menon et al. (2012) reported no relationship between vitamin D levels of patients with asthma and controls. The conflicting reports of these publications may be due to that vitamin D levels were measured only once, which may not be sufficient to make a conclusion in these chronic diseases. Demonstrating vitamin D levels at the peak season of allergy symptoms may be an advantage of our study, as it is known that vitamin D levels vary seasonally (Kasahara et al. 2013).

Vitamin D deficiency was found to be strongly associated with adiposity in the Framingham Heart Study (Cheng et al. 2010). Although Menon et al. (2012) reported no relationship between vitamin D levels of patients with asthma and controls, they detected significantly lower vitamin D levels in both obese patients with asthma and obese controls. To avoid the confluence, we excluded adult subjects with body mass index above 30 kg/m^2 and children with weight above the 95th percentile of age and gender-specific centile curves from the study.

Although the relationship between vitamin D status and allergic diseases has been well established, there are few studies investigating whether vitamin D supplementation is effective in preventing or treating allergic diseases. Despite the frequent observation of protective association between vitamin D and allergy, supplementation was linked with increased risk of allergic diseases (Kull et al. 2006). It was hypothesized that supraphysiologic levels of vitamin D during the foetal development and infancy period may explain these results (Vassallo & Camargo 2010). But recent studies usually report a beneficial effect of vitamin D supple-

mentation in treatment of allergic diseases (Agrawal et al. 2013).

Although ours is a pioneer study demonstrating decreased vitamin D levels in patients with SAC, there are some limitations that should be addressed. We could not demonstrate a distinct relationship between plasma vitamin D and serum IgE levels in patients with SAC. Our inability to demonstrate a significant relationship can be related to the low number of patients involved in our study. In a population-based birth cohort study assessing the relationship between cord blood vitamin D levels and allergic outcomes, both low and high levels of vitamin D were found to be associated with increased total IgE (Rothers et al. 2011). This may be due to the dual effect of vitamin D on both Th_1 and Th_2 cells as discussed above. The second limitation is that we used electrochemiluminescence technique to quantify plasma vitamin D levels. This technique is reported to underestimate vitamin D levels slightly when compared to liquid chromatography–tandem mass spectrometry method (Roth et al. 2008). But we believe that this underestimation should not be important as we analysed the samples of SAC and control groups at the same time and compared the results between the groups. Despite these shortcomings, our preliminary study generates valuable hypotheses and provides a basis for future studies.

In conclusion, we demonstrated lower plasma vitamin D levels in patients with SAC compared with the control group. To conclude a certain association between vitamin D status and allergic conjunctivitis, both multicenter larger case series and further studies investigating the effects of vitamin D supplementation should be performed in the future. According to those results, supplementation of vitamin D might be considered, in case of established deficiency, for the treatment of patients with allergic conjunctivitis in addition to the classic therapy.

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