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Study Effect of Vitamin D on the Immunopathology Responses of the Bronchi in Murine Model of Asthma

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ABSTRACT

Allergic asthma is a complicated respiratory problem characterized by airway inflammation, airway hyperresponsiveness (AHR), breathlessness, mucus hyper-secretion, and goblet cell hyperplasia. Asthma is controlled by genetic and environmental factors. Allergy is the main trigger of asthma and is mediated by Th2 cytokines along with IgE production. Vitamin D (Vit D) is the main supplementary factor for the immune system. In the present study, we investigated the effect of Vit D on the exacerbation of allergic asthma.

A murine model of allergic asthma was induced by ovalbumin (OVA) in four of five groups of studied female BALB/c mice (each group, n=20). One group was considered as control. Of OVA-induced mice, two groups received Vit D via oral (10,000 IU/kg diet) or intranasal (inhalation) forms (30 min on days 25, 27, and 29), and the third group received budesonide. At least, AHR, the levels of IL-4, IL-5, IL-13, and INF-γ in bronchoalveolar lavage fluid (BALF), serum IgE and histamine, *IL-25* and *IL-33* gene expression, as well as histopathology study of the lung were done.

The Penh values, type2 Cytokines in BALF (in both protein and molecular levels), total IgE and histamine, perivascular and peribronchial inflammation, goblet cell hyperplasia, and mucus hypersecretion decreased significantly in both oral and intranasal Vit D-treated asthmatic mice groups, especially on day 38 of orally treated mice.

Here, we found Vit D as a promising agent in control of allergic asthma with a remarkable ability to decrease the severity of inflammation. Therefore, Vit D sufficiency is highly recommended in asthmatic patients.

Keywords: Asthma; Allergy and immunology; Vitamin D

INTRODUCTION

Allergic asthma is a complicated chronic respiratory disease with a higher prevalence in the world especially in industrial countries. Asthma is characterized by eosinophilic inflammation of the airway, airway hyperresponsiveness (AHR), bronchoconstriction, breathlessness, recurrent smooth muscle spasm, mucus hyper-secretion, and goblet cell hyperplasia with recurrent episodes of dyspnea,

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coughing, wheezing, and chest pain. Allergic asthma mostly occurs in younger adults and is a major cause of morbidity and mortality afflicting more than 350 million individuals globally.[1,2]

Asthma is under control of genetic and environmental factors and genetic predisposition leads to polarization of Th lymphocyte cells to the Th2. Allergy is the main trigger of asthma pathophysiology and is mediated by Th2 (Type-2) cytokines (IL-4, 5, and 13). In atopic patients, IgE is produced from plasma cells which are induced by allergens, under the force of Type-2 cytokines. Produced IgE binds to IgE-receptors (FcεR) on the surface of mast cells. Subsequently, re-exposure to an allergen causes the formation of the allergen-IgE complex on the surface of the mast cells that leads to release of the stored mediators in the mast cell granules and then, synthesis and release of other mediators which lead to airways inflammation and bronchoconstriction. Also, eosinophils are the main cells in asthma pathophysiology that can be activated by IL-5 and lead to eosinophilic inflammation in the airways of the asthmatic lung.[3,4]

Unfortunately, available anti-asthma drugs cannot completely cure asthma. Therefore, controlling asthma immunopathology is the main strategy to prevent asthma attacks and control harmful mechanisms of disease.[1,2,4]

Food supplementary may be an important environmental factor in the initiation of allegro-immunopathology and Vit D as one of the main food supplementation, is a critical and main complementary factor for bioactivity of the cells. Vit D3 can come from the diet and also, in the skin, 7-Dehydrocholesterol (7-DHC) is converted to pre-VitD3 underexposure to UVB rays, and then transformed to Vit D3 (cholecalciferol) by a thermally induced isomerization. Vit D3 undergoes hydroxylation to 25-hydroxyvitamin D3 (25OHD) in the liver, then is hydroxylated in the kidney to has biologically active form 1,25-dihydroxy vitamin D3 [1,25(OH)2D or Vit D or calcitriol].[5]

Some studies showed that Vit D insufficiency (25OHD levels of less than 30 ng/mL or 75 nmoL/L) has contributed to asthma and allergic disease rising. [6,7] This fact can highlight the potential risk of Vit D deficiency which increases the allergic disease rates worldwide. Immunomodulatory effects of Vit D on allergen-induced inflammatory pathways are acted by VDR expression on a variety of immune cells. Vit D may balance Th1/Th2 cytokines in response to various pathogens and allergens and can inhibit Th2 responses by suppressing the production of IL-4 and IL-13.[7-9]

The major aims of allergic asthma treatment and control are focused on the prevention of eosinophilic inflammation, mucus hypersecretion, bronchoconstriction, and airway remodeling. At present study, we planned to use vitamin D in food (oral administration) of a murine model of asthma to investigate the impact effect of Vit D on exacerbation of allergic asthma symptoms with monitoring of Vit D level in serum.

MATERIALS AND METHODS

Animal

Female BALB/c mice (6 weeks old) were purchased from the Pasteur Institute (Iran) and for adaptation to the new place, acclimatized under the standard laboratory conditions (24±2°C temperature, 50±10% humidity, 12 h light-dark cycle, and also, pathogen-allergen free) for 1 week.

The studies involving animals were reviewed and approved by the ethics committee of the animal house of ix.med.vet.dep, 2020 (No. IX.MED.VET.DEP.REC.2020.140007.2).

Animal Sensitization and Treatment Schedule

A total of 100 mice were allocated in five groups (n=20). In four groups, the airway allergic inflammation was induced by ovalbumin (Sigma-Aldrich, USA) according to a previously described protocol. [2] Briefly, to produce a murine model of allergic asthma, the mice were sensitized by intraperitoneal injection of 20 µg OVA with 50 µL aluminum hydroxide (Alum) (Sigma-Aldrich, Netherlands) as an adjuvant dissolved in 1 ml normal saline at day 1 and also boosting injection was applied at day 14. Then, the mice were challenged by inhalation of 1% OVA solution aerosolized for 30 min/day by an ultrasonic nebulizer (NE-U07, Omrom, Japan) on days 24, 26, 28, and 30 (Figure 1). The fifth group was sensitized and challenged only by PBS (negative control group). One of the OVA groups received Vit D by oral administration of Vit D-containing food (vitamin D supplemented 10,000 IU/kg diet)[10] on days 25, 27, and 29 (asthmatic treated group).

The second OVA group received Vit D by inhalation of Vit D solution by nebulizer on days 25, 27, and 29 for 30 min. One other asthmatic group received a normal diet (positive control group) and the last asthmatic group was treated with budesonide on days 25, 27, and 29 (standard drug control group). In the end, the samples, bronchoalveolar lavage fluid (BALF), blood, and lung tissue were collected on days 31 and 38, each time by euthanizing of 5 mice (other remaining 5 mice in each group were used for AHR measurements).

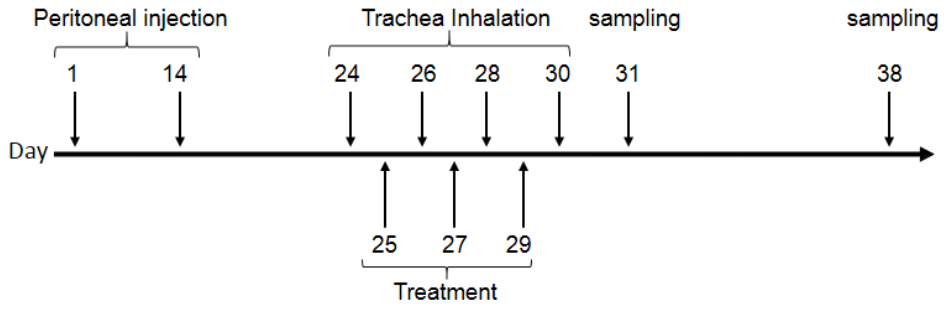

Figure 1. Asthma model animal producing. Mice were sensitized by intraperitoneal injection of 20 µg ovalbumin (OVA) with alum adjuvant on days 1 and 14. The sensitized mice were challenged by intratracheal inhalation of 1% OVA solution that was aerosolized by ultrasonic nebulizer on days: 24, 26, 28, and 30 for 30 min per day to produce an asthmatic model.

Methacholine (MCh) Challenge Test

The MCh challenge test is used to determine the AHR. AHR was measured on days 30 and 38 in a manner described earlier. [2] Briefly, AHR is assessed by determining enhanced pause (Penh value). After anesthetizing, the mice were tracheotomized and a catheter placed in the trachea of the mice, then connected to a mechanical ventilator. Healthy mice were exposed to PBS aerosols to obtain the baseline Penh value, whereas asthmatic mice were exposed to aerosolized methacholine with a series of doubling concentrations [0 (PBS), 1, 2, 4, 8 and 16 mg/mL].

Measurement of Cytokines Levels in BALF

The levels of IL-4, IL-5, IL-13 as Th2 cytokine and INF-γas Th1 cytokine in BALF were measured using bio-plex mouse cytokine, chemokine assays (Bio-Rad, Nederland) as described before. [2]

IgE Levels in Serum

Before euthanizing the mice, blood samples were collected and serum was separated, then total IgE levels in serum were measured by ELISA kit (BD Biosciences, USA).

Determining the Histamine Level in Plasma

To determine the histamine level in plasma of the studied mice, 30 min after the last challenge, blood samples were taken from the tail vein and after separation of plasma, histamine level was measured by mouse histamine ELISA Kits (Biocompare, USA).

Quantitative Real-time PCR

From BAL cells, total RNA was isolated and reverse transcribed to first-strand cDNA using a cDNA synthesis kit (Maxima First Strand cDNA Synthesis Kit, Thermo Scientific, USA). Quantitative PCR was performed; using a Rotor-Gene SYBR Green PCR Kit (Qiagen, Hilden, Germany).[2] Primers for the two target genes (*IL-25* and *IL-33*) and one primer-pair for *GAPDH* as a reference gene were 5'-3'; IL-25 Forward: CTCAACAGCAGGGCCATCTC, Reverse: GTCTGTAGGCTGACGCAGTGTG; IL-33, Forward:TCCTTGCTTGGCAGTATCCA, Reverse: TGCTCAATGTGTCAACAGACG and GAPDH Forward: TGTTCCTACCCCCAATGTGT, Reverse: GGTCCTCAGTGTAGCCCAAG.

Histopathology

At the end of each two periods (day 31 and 38), mice lung tissues were isolated and after fixation in formalin solution, trimmed and paraffin-embedded. The sections were stained with H&E and AB-PAS stain. Afterward, the peribronchiolar and perivascular inflammation, mucus hyper-secretion, and goblet cell hyperplasia in the airways were evaluated by using a

point scoring system as described before. [2] In brief, histopathological sections of the lung tissue were examined by two pathologists in five repeats by each pathologist in 10 randomly selected microscopy fields on sections at 400x magnification. Goblet cells number was quantified according to GCI. Moreover, peribronchiolar and perivascular inflammation was examined according to the incomplete or complete layer of Eos around bronchia or vessels. Mucus hypersecretion was measured according to that what percentage of the airway has been obstructed by mucus.

Statistical Analysis

All experiments were repeated three times and the result has been reported as means ± SD. For analysis, SPSS (version 19) has been used and analyses were performed by using GraphPad Prism (version 6). Correlation analysis was carried out using Pearson's method. Also, the paired t-test was used to analyze methacholine challenge from the baseline. The differences between treated and nontreated asthmatic groups and control groups and the differences between treated asthmatic groups and non-treated asthmatic groups were analyzed using unpaired Student's t-tests. The *p*-value less than 0.05 was supposed to be significant.

RESULTS

AHR

The Penh values of AHR were significantly ($p<0.05$) increased in the asthmatic group (on day 38 for 4 mg/mL MCh: 6.5±0.2) compared with the negative control group (in day 38 for 4 mg/mL MCh: 1.2±0.1) for all concentrations of MCh (Figure 2). Vit D-oral (in day 38 for 4 mg/mL MCh: 4.0±0.1) and budesonide (in day 38 for 4 mg/mL MCh: 2.5±0.2) received asthma groups displayed a significant ($p<0.05$) reduction of the Penh value mean compared with the non-treated asthmatic mice and Vit D-inhaler (in day 38 for 4 mg/mL MCh: 6.0±0.1) received asthmatic mice displayed no significant difference ($p>0.05$) in reduction of the Penh value for all concentrations of methacholine in day 38.

Cytokines Levels

The levels of IL-4 (81.97±2.17 and 80.37±2.43 pg/ml respectively on day 31 and 38), IL-5 (74.21±1.99 and 75.19±2.04 pg/mL respectively on day 31 and 38), and IL-13 (132.95±2.14 and 156.45±2.01 pg/mL respectively on day 31 and 38) were increased in OVA group as compared to healthy group (IL-4: 42.11±1.01 and 43.28±1.36, IL-5: 38.52±1.29 and 37.14±0.98, and IL-13: 67.21±1.41 and 69.41±1.71 pg/mL respectively)and a reverse trend was found in IFN-γ (asthma group: 23.25±0.95 and 19.24±2.14, healthy group: 51.02±2.01 and 50.21±2.36 pg/mL respectively on day 31 and 38) ($p<0.05$) (Figure 3). In the two treated groups (Vit D-oral received group and budesonide), a reverse trend was found and significantly reduced IL-4, IL-5, IL-13, and restored the IFN-γ levels on day 31 and 38 ($p<0.05$). IL-13 on day

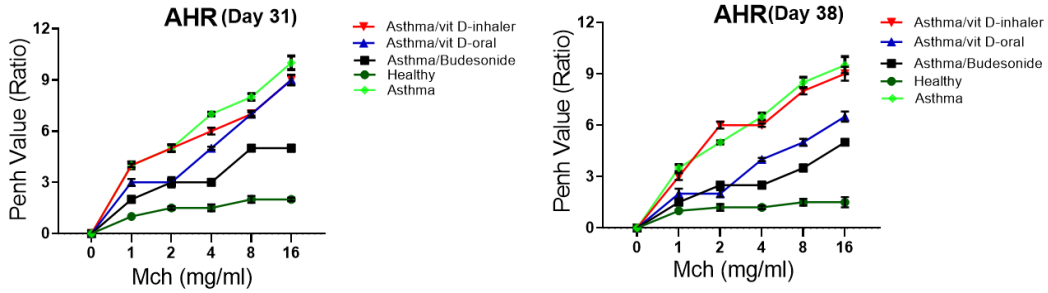

Figure 2. BALB/c mice (6 weeks old) were allocated in five groups (n=20) that include; negative control group, and four allergic asthma groups that received Vit D by oral, Vit D by inhalation, budesonide and no-treatment. For determining Penh value to study airway hyperresponsiveness (AHR) in response to increasing doses of methacholine (MCh); Mice after anesthetization were tracheotomized. Mice were exposed to a doubling concentrations series of aerosolized MCH (1, 2, 4, 8, and 16 mg/ml) to obtain AHR changes. The Penh values were increased in the asthmatic mice compared with healthy mice for all concentrations of MCh. Ovalbumin (OVA)-sensitized and Vit D received group by oral had low Penh value in comparison to OVA-sensitized group in day 38. The healthy group had minimum Penh values. P<0.05 was considered as significant difference.

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31 in the Vit D-inhaler received group 102.11±2.44 pg/mL was decreased significantly compared to asthma group132.95±2.14 pg/mL (p<0.05).

IgE

The total IgE level in serum was significantly increased in the OVA group on days 31 and 38 (2199.85±4.36 and 2247.95±5.98 ng/mL respectively) compared to the negative control group on days 31 and 38 (38.71±1.51 and 56.17±1.39 ng/mL respectively) p<0.05). However, on days 31 and 38, OVA and Vit D-oral received group (1821.97±3.25 and 672.98±4.21 ng/ml respectively) and OVA-budesonide group (1420.27±2.08 and 721.95±2.21 ng/mL respectively) significantly reduced the rise of total IgE levels in serum (p<0.05) (Figure 4). On days 31 and 38, OVA and Vit D-inhaler received group (1994.87±3.29 and 2001.48±4.98 ng/mL respectively) reduced the rise of total IgE levels in serum compared to the OVA-sensitized group, but was not significantly decreasing.

Histamine Level

The histamine level was significantly increased in all OVA-challenged groups on day 31 which was decreased on day 38 (Figure 4). However, on day 31, OVA groups that were received Vit D-oral, Vit D-inhaler, and budesonide significantly reduced the rise of histamine levels compared to the OVA group (401±1.6, 552±2.2, 387±1.1, and 625.2±3.9 ng/mL respectively) (p<0.05). On day 38, OVA groups that were received Vit D-oral and budesonide significantly reduced the rise of histamine levels compared to the OVA group (184.6±1, 211.9±1.5, and 421.3±2.4 ng/mL respectively) and OVA and Vit D-inhaler received group was not significantly decreasing compared to the OVA group (p<0.05).

Real-time PCR

Expression of IL-25 (2.21±0.28 and 1.09±0.15 respectively) and IL-33 (3.68±0.27 and 2.45±0.52 respectively) mRNA were decreased in the OVA Vit D-oral group compared to the positive control group (IL-25: 4.00±0.53 and 5.00±0.27, IL-33: 8.00±0.5555 and 7.00±0.27 respectively) on days 31 and 38. Expression of IL-25 mRNA (3.10±0.53) was decreased in the OVA Vit D-inhaler group compared to the positive control group on day 38 (Figure 4) (p<0.05).

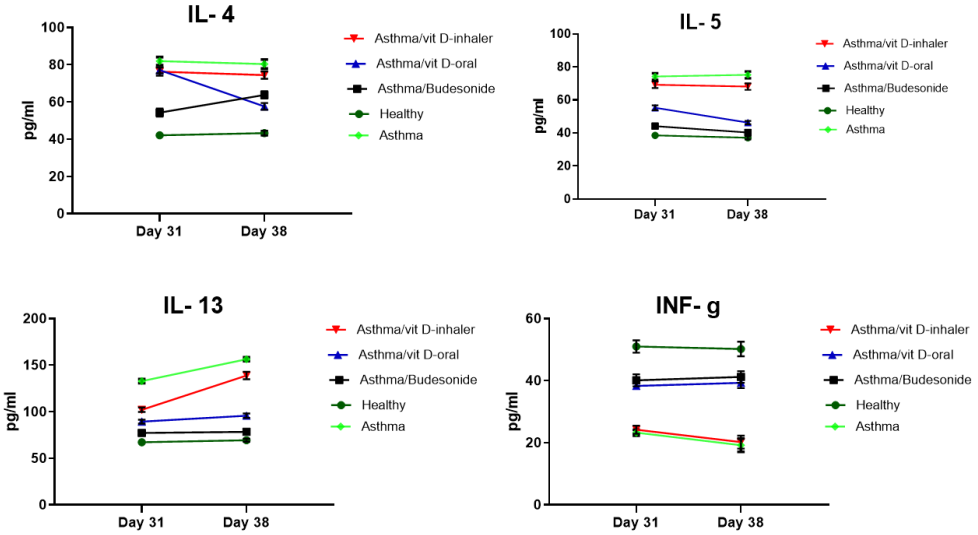

Figure 3. BALB/c mice (6 weeks old) were allocated in five groups (n=20) that include; negative control group, and four allergic asthma groups that received Vit D by oral, Vit D by inhalation, budesonide and no-treatment. To evaluate Bronchoalveolar lavage fluid (BALF) cytokines levels; the levels of IL-4, IL-5, and IL-13 in BALF were increased in Ovalbumin (OVA) group, and that was reduced by Vit D oral administration, and a reverse trend was found in IFN-γ level. p<0.05 was considered as significant difference.

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Histopathology

Perivascular and peribronchial inflammation (eosinophilic inflammation) were not significantly decreased in OVA groups that were received Vit D-oral and Vit D-inhaler compared to the OVA group on day 31 ($p>0.05$), but peribronchial inflammation was significantly decreased in the OVA group that was received Vit D-oral on day 38 (2.50±0.30) compared to the OVA group (3.50±0.20) and also, perivascular inflammation was significantly decreased in OVA groups that were received Vit D-oral and Vit D-inhaler (2.40±0.10 and 3.00±0.30 respectively) compared to OVA group (3.70±0.10) on day 38 ($p<0.05$) (Figure 5).

Goblet cell hyperplasia and mucus hypersecretion were significantly induced in the airways of the asthmatic group compared to the healthy group ($p<0.05$). The stained airway epithelium was greater in the asthmatic group than in the control group (Figure 6). On days 31 and 38, mucus hyper-secretion was significantly decreased in the OVA group that was received Vit D-oral (2.80±0.05 and 1.60±0.04 respectively) compared to the OVA group (3.90±0.09 and 3.70±0.04 respectively).

Goblet cell hyperplasia was significantly decreased in the airway of OVA-challenged mice that were received Vit D-oral and Vit D-inhaler (3.30±0.10 and 3.10±0.30 respectively) compared to the OVA group (3.90±0.10) on day 38 ($p<0.05$) (Figures 5 and 6).

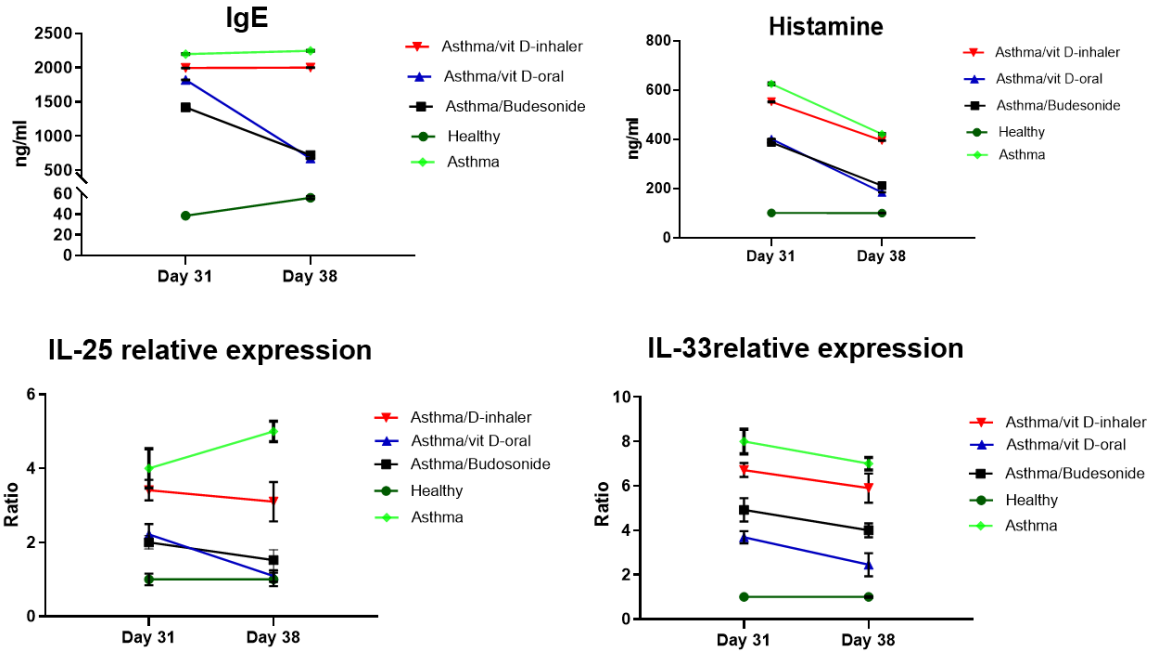

Figure 4. BALB/c mice (6 weeks old) were allocated in five groups (n=20) that include; negative control group, and four allergic asthma groups that received Vit D by oral, Vit D by inhalation, budesonide and no-treatment. The total IgE and histamine levels in serum and *IL-25* and *IL-33* gene expression were studied. In the collected blood samples of the ovalbumin (OVA) group, total IgE and histamine levels were increased. Total IgE and histamine levels in serum of Vit D treated mice have been controlled. Also, *IL-25* and *Il-33* gene expression in the Vit D treated mice has been reduced. P<0.05 was considered as significant difference.

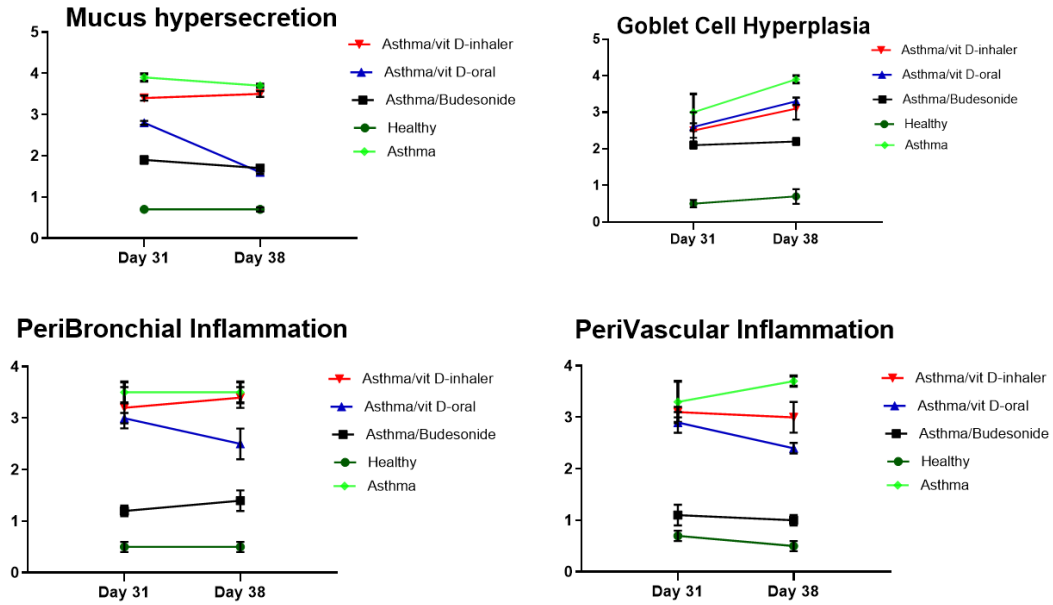

Fig. 5. BALB/c mice (6 weeks old) were allocated in five groups (n=20) that include; negative control group, and four allergic asthma groups that received Vit D by oral, Vit D by inhalation, budesonide and no-treatment. Perivascular and peribronchiolar inflammation, goblet cell hyperplasia, and mucus hyper-secretion were studied in lung sections. Inflammation was increased in asthma groups and Vit D can control eosinophilic inflammation in asthmatic mice. Increased mucus secretion and goblet cell hyperplasia were decreased in Vit D received mica as compared to the ovalbumin (OVA) group. Perivascular and peribronchial inflammation were not significantly decreased in Vit D received groups on day 31 ($p>0.05$), but peribronchial inflammation was significantly decreased in Vit D-oral received group on day 38 ($p<0.05$) and also, perivascular inflammation was significantly decreased in Vit D-oral and -inhaler received groups ($p<0.05$) on day 38. $P<0.05$ was considered as significant difference.

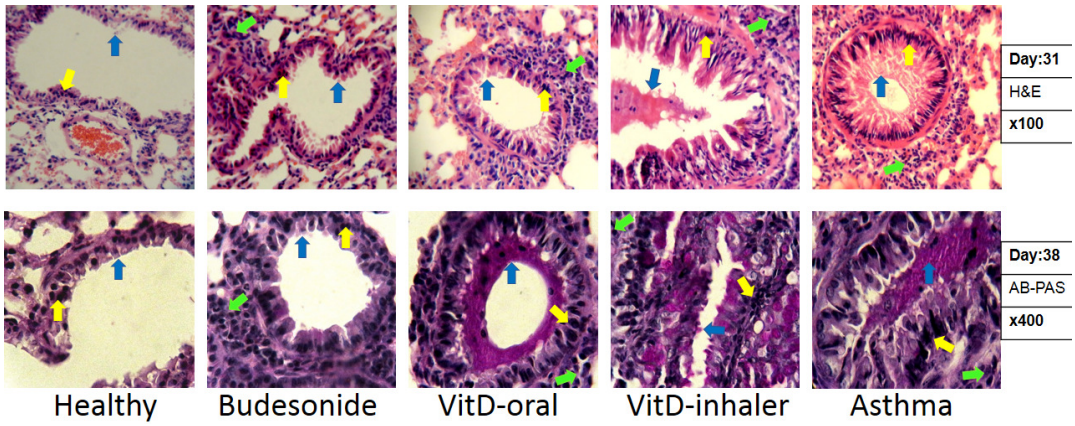

Figure 6. Histopathology of lung sections: Lung tissues after fixation were stained with two H&E and AB-PAS stains. Afterward, the inflammation of the perivascular and the peribronchiolar, hyperplasia of the goblet cell and mucus hypersecretion were evaluated in 10 randomly selected fields of the microscopy sections at 400x magnification (blue arrows: mucus secretion, yellow arrows: goblet cells, and green Arrows: peribronchiolar inflammation)

DISCUSSION

Since today's hectic and stressful lifestyle, people have increasingly been faced with some immune system-related diseases. In this regard, allergic diseases such as allergic asthma seem to be raised alarmingly. Allergy and Asthma are a chronic and prevalent disease which is generally developed due to some malfunctions in the immune system. However, there is no complete cure for these until recently. In fact, in all countries, a heavy economic burden is imposed on the national budget annually for providing healthcare services for allergy and asthma patients. In mentioned cases, asthmatic exacerbations and allergies are the major problems reducing the life quality of patients and imposing high economic costs on the national healthcare system. Vitamins' effects on the immune system may be important in the development and severity of allergic diseases (asthma, eczema, and food allergy). Vit D as an essential supplementary agent for most body physiological activity can modulate responses of the innate and adaptive immune system and may influence the development and the initiation of allergic diseases. Therefore, in this study, the effect of Vit D on allergy-related biological factors was studied. While there is growing observational and experimental evidence for the regulatory and modulatory role of Vit D on the immune system, in normal or pathological conditions. We evaluated to determine whether supplementation of vitamin D affects allergic bio-factors in allergic asthma disorder. So, after producing an allergy asthma animal model, the effect of Vit D on the animal model was surveyed.

Vit D is found mainly in foods and can be produced in the skin under the sunshine. [10] In this study, we have evaluated the effect of Vit D in an animal model of allergic asthma and used a safe dose of Vit D (which did not reach toxicity dose). In the present study, we found that Vit D acts as an immune modulator in allergic pathophysiology and can be an effective agent to control allergic reactions, AHR, and inflammatory responses in asthmatic mice, especially in oral administration. Therefore, Vit D may prevent asthma attacks over a long period and prevent lung injury. After passing time, the beneficial effect of Vit D was better than the previous time and on day 38, it had a stronger effect than day 31. Although we could not find sharp changes in the Vit D-inhalation group, this result was sharp and significant in Vit D-oral received asthma group.

In the asthmatic group of our study, AHR was increased compared to healthy mice. The level of AHR was lower in the OVA-Vit D-treated group in comparison to the OVA group. These results showed that Vit D worsens the airway hyper responses in asthmatic mice. Previous studies showed that Vit D promotes calcium absorption and has stimulatory effects on the neuromuscular system and immune cell functions. Also, it has an inhibitor effect on the differentiation and maturation of dendritic cells, which leads to a tolerogenic state with increasing IL-10 production.[11-13] Similarly, Vit D supplementation reduced AHR and inflammation in the lung of the allergic asthma mouse. [10,14,15] The Penh values of AHR were significantly increased in the asthmatic group compared with the negative control group ($p<0.05$) for all concentrations of MCh. OVA and Vit D-oral received group and asthmatic mice that were treated with budesonide, displayed a reduced mean Penh value compared with the response of the asthmatic mice and asthmatic Vit D-oral received mice ($p<0.05$) for all concentrations of MCh on day 38. A study by Agrawal et al, 2013 showed that Vit D deficiency is associated with higher AHR in asthmatic mice than those in Vit D sufficient mice and also, was a similar result for airway remodeling, high BALF eosinophilia, increased Th2 cytokines.[14] In addition, we found that the levels of key cytokines involved in Th2 responses and its products that are related to allergy and asthma pathophysiology mechanisms such as IgE in the OVA group were increased in comparison to the healthy group and Vit D-oral treated asthma group was close to the levels in budesonide treated asthma group, especially after passing time.

In the allergic reaction, Vit D has an anti-proliferative effect on B cell a and promotes the apoptosis of B cells.[16,17] It can reduce IgE production during allergy and asthma attacks.

Vit D plays a significant part in healthy immunity and disease immunopathology; however, it is not completely understood and over 900 genes are regulated by Vit D. Several studies showed that Vit D decreases the creation of Th1 cytokines and can inhibit IL-4 production.[18-21] IL-4 is the main cytokine in the pathology of allergic diseases and Vit D can harness this cytokine, therefore, Vit D can act as an anti-allergy

agent. The levels of IL-4, IL-5, and IL-13 in BALF and the total IgE level in serum were increased in the asthma group as compared to the healthy group and a reverse trend was found in IFN-γ. In the Vit D-oral received group, IL-4, IL-5, IL-13 and IgE were significantly reduced and restored the IFN-γ levels. Also, Vit D can control histamine levels in allergic reactions. These changes showed that Vit D can control allergic reactions and asthma pathophysiology in studied mice. Moreover, increased levels of Th1 cytokine (INF-γ) and decreased levels of Th2 cytokines (IL-4, 5, and 13) in the OVA Vit D-treated group were significantly changed, in comparison to the OVA group. Our results indicate that Vit D can act as a strong agent for control of the allergic responses and inflammation in the airways. It can inhibit exacerbation of the inflammatory allergic symptoms in the asthmatic airway and lead to a controlling of allergic asthma.

Interestingly, differentiation of the Th2 cell can be inhibited by Vit D.[11] It could be the main pathway to control and harness type-2 cytokines network responses.

The role of Vit D in asthma is not yet clear, but some studies showed that intake of Vit D during pregnancy influences children and adults asthma.[22] A survey on 75 Italian asthmatic people found that the prevalence of Vit D-deficiency was 53.3% and 17% of asthmatics people in North America had Vit D deficiency. A cohort study revealed that low levels of 25 (OH) D in serum predicted asthma-associated symptoms.[23-25] But a study in New Zealand revealed that low serum 25 (OH) D levels were not associated with the incidence of asthma.[26] It has been suggested that a westernization lifestyle leads to Vit D deficiency. Since, the majority of the population spends time away from sun exposure, which leads to Vit D deficiency. [27] A study by Brehm, et al conducted the relations between asthma exacerbations and serum Vit D levels in 1024 asthmatic children and confirmed that levels of Vit D<30 ng/mL had increased asthma exacerbations risk and children with Vit D insufficiency had higher asthma exacerbation chances.[22] Another study by Shahin, et al. conducted that in asthmatic patients, serum Vit D level was significantly decreased (19.88±9.6 ng/mL) as compared to the healthy people (33.5±6.1 ng/mL).[28] Eosinophilic perivascular and peribronchial inflammation, goblet cell hyperplasia, and mucus hypersecretion were significant may be regulated by Vit D and these effects can be enhanced in continuous time.

Vit D has genomic effects on fetal lung development and types II fetal alveolar epithelial cells express VDR, So, pulmonary maturation is responsive to Vit D exposure. *TXNIP*, *SCRAB2*, *PIP5K1B*, and *LAMP3* are Vit D-related genes that have a role in fetal lung development and are significantly overexpressed in cells derived from asthmatic children (the link between Vit D pathway genes and asthma).[29,30] Vit D potentially decreases asthma and allergy severity through a variety of mechanisms, include; immune cells regulation, improved lung function with reducing inflammatory responses, airway smooth muscle relaxation, reduced airway smooth muscle mass, and remodeling and also, reversal of steroid resistance with produced IL-10 and modifying down-regulation of glucocorticoid receptors. Vit D can amplify glucocorticoid induction of MPK-1 and IL10 that are critical for anti-inflammatory and immunosuppressive responses.[10,28,31-33] Bosse et al showed that Vit D increases glucocorticoid bioavailability in smooth muscle cells of the bronchi. [34] In the current study, expression of *IL-25* and *IL-33* gene was decreased in the OVA VitD-oral group and Vit D may have an effect on gene expression on Th2 upper-hand cytokines such as *IL-25* and *IL-33* and can act as the main immunomodulatory factor. Also, Vit D may regulate epigenetic events, which promote allergic responses.

Vit D due to induction of FOXP3+ regulatory T cells inhibits Th2 responses. The anti-inflammatory and anti-allergy protective role of Vit D may be caused by the suppression of NF-kB and TGF-β/SMAD signaling pathways. Together with the Nrf2/HO-1 pathway activation, Vit D may be able to protect against oxidative injury and could control asthma.[35,36] Another report showed that Vit D is important to maintain the mast cell stability with inhibition of IgE-sensitized mast cell activation. Vit D may interfere with the adaptor protein Myd88 and the FcεR1β subunit (two important key components of mast cell activation) of the high-affinity IgE receptor.[37]

However, there are several limitations to this study. Firstly, we did not evaluate the toxic dose effect of Vit D in allergic asthma. We have just examined its effects due to the normal range administered. Next, we used an animal model to study and we could not study in human subjects. Last, we could not determine the level of other related bio-factors in this study.

Our study focused on immunoregulation of Vit D on the pathophysiology of allergic mechanisms of asthma which can be administered in asthmatic patients with Vit D deficiency. The promising results in an animal study and further human study can direct us to plan for the commercialization of the treatment. The positive results from animal studies help us to design further studies to use the treatment in human patients to reduce the allergy and asthma symptoms and decrease the costs of healthcare and increasing the life quality. In the present study, we found that Vit D can be a useful agent in the control of allergic asthma and can decrease the severity of inflammatory responses in asthmatic mice. Therefore, the prevention of Vit D deficiency is strongly recommended for patients with asthma. Asthmatic patients should give more attention to their diet and Vit D levels of their bodies. Also, in allergen-specific immunotherapy, supplementation with Vit D will be able to enhance the beneficial effects of treatment protocols.

CONFLICT OF INTEREST

There are no conflicts of interest.

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REFERENCES

1. Athari SS. Targeting cell signaling in allergic asthma. Signal Transduct Target Ther. 2019; 4:45.
2. Athari SS, Pourpak Z, Folkerts G, Garssen J et al. Conjugated Alpha-Alumina nanoparticle with vasoactive intestinal peptide as a Nano-drug in treatment of allergic asthma in mice. Eur J Pharmacol. 2016; 15(791):811-820.
3. Athari SS, Athari SM, Beyzay F, Movassaghi M, Mortaz E, Taghavi M. Critical role of Toll-like receptors in pathophysiology of allergic asthma. Eur J Pharmacol. 2017; 808(4):21-27
4. Athari SM, Mehrabi Nasab E, Athari SS. Study effect of Ocimumbasilicum seeds on mucus production and cytokine gene expression in allergic asthma mice model. Revue Française d'Allergologie. 2018; 58(7):489-93
5. Mirzakhani H, Amal Al-Garawi, Scott T. Weiss and Augusto A. Litonjua. Vitamin D and the development of allergic disease: how important is it? Clin Exp Allergy. 2015; 45(1) 114-25
6. Litonjua, A. The Role of Vitamin D in the Development, Exacerbation, and Severity of Asthma and Allergic Diseases. Vitamin D and the Lung: Mechanisms and Disease Associations. In: Litonjua, AA, editor. Respiratory Med. 2012; 3(2):201-38.
7. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc. 2013; 88(7):720–55.
8. Adorini L, Penna G, Giarratana N, Roncari A, Amuchastegui S, Daniel KC, Uskokovic M. Dendritic cells as key targets for immunomodulation by Vitamin D receptor ligands. J Steroid Biochem Mol Biol. 2004;89–90(1-5):437–41.
9. Pichler J, Gerstmayr M, Szepfalusi Z, Urbanek R, Peterlik M, Willheim M. 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. PediatrRes. 2002; 52(1):12–8.
10. Kimberly D. Fischer, Sannette C. Hall and Devendra K. Agrawal. Vitamin D Supplementation Reduces Induction of Epithelial-Mesenchymal Transition in Allergen Sensitized and Challenged Mice. PLoS One. 2016;11(2):e0149180.
11. Paul E. Pfeffer, Elizabeth H. Mann, Eve Hornsby, Emma S. Chambers, Yin-Huai Chen, Louise Rice, and Catherine M. Hawrylowicz. Vitamin D Influences Asthmatic Pathology through Its Action on Diverse Immunological Pathways. Ann Am Thorac Soc. 2014;11(5):S314-320
12. Colotta F, Jansson B, Bonelli F. Modulation of inflammatory and immune responses by vitamin D. J Autoimmun. 2017;85(14):78–97.
13. Bscheider M, Butcher EC. Vitamin D immunoregulation through dendritic cells. Immunology. 2016;148(3):227–36.
14. Tanupriya Agrawal, Gaurav K. Gupta, and Devendra K. Agrawal. Vitamin D Supplementation Reduces Airway Hyperresponsiveness and Allergic Airway Inflammation in a Murine Model. Clin Exp Allergy. 2013;43(6):672–83.
15. Karin Hufnagl • Erika Jensen-Jarolim. Vitamin A and D in allergy: from experimental animal models and cellular studies to human disease. Allergo J Int. 2018;27(3):72–8
16. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. Nutrients. 2015;7(4):3011–21.
17. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effectsof 1,25-dihydroxyvitaminD3onhuman Bcelldifferentiation. J Immunol. 2007;179(3):1634–47.

18. Mak G, Hanania NA: Vitamin D and asthma. CurrOpinPulm Med. 2011;17(1):1–5.
19. Chambers ES, Hawrylowicz CM: The impact of vitamin D on regulatory T cells. Curr Allergy Asthma Rep. 2011;11(1):29–36.
20. Brehm JM, Schuemann B, Fuhlbrigge AL, et al.: Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol. 2010;126(1):52–8.
21. Ali N S, Nanji K. A Review on the Role of Vitamin D in Asthma. Cureus. 2017;9(5):e1288.
22. Hollams EM, Hart PH, Holt BJ, et al.: Vitamin D and atopy and asthma phenotypes in children: a longitudinal cohort study. Eur Respir J. 2011;38(6):1320–7
23. Freishtat RJ, Iqbal SF, Pillai DK, et al.: High prevalence of vitamin D deficiency among innercity African American youth with asthma in Washington, DC. J Pediatr. 2010;156(6):948–52.
24. Chinellato I, Piazza M, Sandri M, et al.: Vitamin D serum levels and markers of asthma control in Italian children. J Pediatr. 2011;158(3):437–41.
25. Devereux G, Wilson A, Avenell A, et al.: A case-control study of vitamin D status and asthma in adults. Allergy. 2014;65(5):666–7
26. Camargo CA, Ingham T, Wickens K, et al.: Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. Pediatrics. 2011;127(1):180–7.
27. FemkeBaeke, Tatiana Takiishi, Hannelie Korf, Conny Gysemans, Chantal Mathieu. Vitamin D: modulator of the immune system. CurrOpinPharmacol; 2010;10(4):482-96.
28. Shahin MYA, El-lawah AA, Amin A, et al.: Study of serum vitamin D level in adult patients with bronchial asthma. Egypt J Chest Dis Tuberc. 2017;66(1):5-9.
29. Kho, Alvin T, Sunita Sharma, WeiliangQiu, Roger Gaedigk, Barbara Klanderman, SiminNiu, Chris Anderson, James S Leeder, Scott T Weiss, and Kelan G Tantisira. Vitamin D related genes in lung development and asthma pathogenesis. BMC Med Genomics. 2013;6(1):47.
30. Marin L, Dufour ME, Nguyen TM, Tordet C, Garabedian M. Maturational changes induced by 1 alpha,25-dihydroxyvitamin D3 in type II cells from fetal rat lung explants. Am J Physiol. 1993; 265(1 pt 1):L45–52.
31. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, Adikibi T, Pridgeon C, Dallman M, Loke TK, Robinson DS, Barrat FJ, O'Garra A, Lavender P, Lee TH, Corrigan C, Hawrylowicz CM. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. J Clin Invest. 2006;116(1):146–55.
32. Majak P, Olszowiec-Chlebna M, Smejda K, Stelmach I. Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. J Allergy Clin Immunol. 2011;127(5):1294–6.
33. Li F, Peng M, Jiang L, Sun Q, Zhang K, Lian F, Litonjua AA, Gao J, Gao X. Vitamin D deficiency is associated with decreased lung function in Chinese adults with asthma. Respiration. 2011; 81(6):469–75.
34. Bosse Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. PhysiolGenomics. 2007; 29(2):161–8.
35. Wang Z, Zhang H, Sun X, Ren L. The protective role of vitamin D3 in a murine model of asthma via the suppression of TGF-beta/Smad signaling and activation of theNrf2/HO-1 pathway. Mol Med Rep. 2016;14(3):2389–96.
36. David A Jolliffe, Lauren Greenberg, Richard L Hooper, Christopher J Griffiths, Carlos A Camargo Jr, Conor P Kerley, Megan E Jensen, David Mauger, Iwona Stelmach, Mitsuyoshi Urashima, Adrian R Martineau. Vitamin D supplementation to prevent asthma exacerbations: a systematic review and meta-analysis of individual participant data. Lancet Respir Med. 2017;5(11):881–90
37. Liu ZQ, Li XX, Qiu SQ, Yu Y, Li MG, Yang LT, et al. Vitamin D contributes to mast cell stabilization. Allergy. 2017;72(8):1184–92.