

Reduction of respiratory infections in asthma patients supplemented with vitamin D is related to increased serum IL-10 and IFN γ levels and cathelicidin expression

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ABSTRACT

Background: Vitamin D is a molecule that modulates the immune response and shows anti-inflammatory effects that are beneficial for the control of chronic diseases such as asthma. The trial aim was to explore the effect of vitamin D supplementation on the colonization of pathogenic bacteria in the upper respiratory tract of allergic asthmatic patients.

Methods: This study was conducted in 86 patients between 18 and 50 years of age who were randomly divided into two groups. Both groups received the treatment recommended by the Global Initiative for Asthma (GINA). One group also received calcitriol (1,25-(OH) $_2$ D $_3$), and the other group received a placebo. At baseline and 6 months, skin prick tests were conducted, pharyngeal bacterial cultures were performed, and cathelicidin LL-37 was measured in sputum. Serum levels of IgE, eosinophils, IL-5, IL-9, IL-10, IL-13, and IFN γ were quantified at the beginning and the end of the study.

Results: Serum levels of IL-10 and IFN γ increased significantly in the group of patients with vitamin D supplementation, while IL-5, IL-9, and IL-13 decreased significantly. At the end of the trial, IgE and eosinophil levels significantly decreased but allergen sensitivity did not show any changes from baseline. Respiratory infections were drastically reduced, and this decrease was related to the number of patients who had high serum levels of IL-10 and IFN γ and expressed LL-37 in their sputum.

Conclusion: Treatment of asthma patients with vitamin D reduced respiratory infections, and this effect was related to the increase of cathelicidin LL-37.

1. Introduction

Asthma is a heterogeneous disease characterized by chronic inflammation of the airways and by respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough [1]. Patients with asthma have recently been classified into different phenotypes, which are further subdivided into several endotypes [2]. These endotypes involve considerable damage to the epithelial barrier and severe inflammation of the respiratory mucosa, decreased production of antimicrobial peptides [3], and increased susceptibility to colonization and infection of the respiratory system by pathogenic microorganisms [4]. The severity of asthma depends on characteristics inherent to the patient and the frequency of symptoms, as well as the response to treatment [5]. There are therapeutic options for treatment; however,

the exacerbation is a significant problem in asthma [6]. Asthma exacerbations are often triggered by multiple factors that can act in an additive or synergistic manner. Factors that cause exacerbations include personal habits, environmental conditions, and infectious agents such as viruses and bacteria [6]. Several studies have reported that the bacterial flora in the airways changes dramatically as a result of chronic inflammation in asthma patients [7,8]. Asthma is a chronic disease, and patients suffer a significant decline in quality of life due to their limited ability to perform physical activity [9]. Thus, treatments focus on maintaining respiratory capacity, and it is important to limit exacerbations due to microbial infections [10]. Numerous studies have reported an association between vitamin D deficiency and susceptibility to microbial infections in patients with asthma [11,12]. This nutrient can be metabolized by airway cells and it induces immunomodulatory

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effects by controlling the expression of cytokines, the production of antimicrobial peptides, the differentiation of dendritic cells, and the activation of T cells [13]. Supplementation with this vitamin increases levels of the anti-inflammatory cytokine interleukin-10 (IL-10) [14] and can reduce the use of inhaled corticosteroids in asthmatic patients [15], thereby reducing exacerbations [16]. Although vitamin D supplementation has proven to be effective as add-on therapy to the treatment of asthma and other diseases, some studies of this type of therapy have not produced positive results, likely due to the ethnicity [17], nutritional status [2], and even skin color [18] of the population. In this randomized trial, we explored how vitamin D supplementation during the treatment of adult asthma patients with a specific phenotype characterized by high allergen-specific IgE levels alters the development of the disease and the incidence of bacterial infections.

2. Material and methods

2.1. Participants and interventions

A randomized, double-blind, placebo-controlled trial was conducted in a parallel manner with treated and placebo groups. Patients from the Department of Allergy and Immunology at the General Hospital of Mexico were enrolled. A total of 86 patients from October 2014 until March 2015 due to the greater incidence of bacterial infections in patients with allergic asthma reported for these months in Mexico City, they were selected by simple random sampling, and then randomly allocated to two groups by block randomization; however, only 83 patients completed the 6-month follow-up period (Fig. 1). Both the patients and the staff who administered the drug were blinded to the treatment assignment. This study was approved by the local Research and Ethics Committee of the General Hospital of Mexico following the principles on lined in the Declaration of Helsinki for research in humans (research project number: DI/14/309/04/003).

The inclusion criteria were: (I) a confirmed diagnosis of asthma by a qualified allergist according to the clinical characteristics established by GINA, (II) a clinical history of bacterial infections in the upper respiratory tract recurring for at least 42 days per year, (III) high levels of IgE (above 90 IU/ml) (IV) and peripheral eosinophilia (greater than $0.3 \times 10^3/\mu\text{l}$). Patients with parasitic infections, current antibiotic treatment, hypokalemia, lithiasis, autoimmune disease, pregnancy, immunodeficiency, cancer, and immunosuppressive therapy were excluded. After the explanation of the study protocol, written informed consent was obtained from each selected patient. All participants in this clinical trial received a combined treatment of a beta 2 agonist (salbutamol at rescue dose) and an inhaled anti-inflammatory steroid (beclomethasone 800 $\mu\text{g}/\text{day}$). During this protocol, patients went to

the department every two months, and their visits included a clinical inspection. During the examination, direct symptoms (related to the respiratory system) and general symptoms were recorded. After symptoms of bacterial infection (tonsillitis, pharyngitis, tracheitis, laryngitis, pneumonia) were recorded and the diagnosis was confirmed by bacteriological cultivation, a specific antimicrobial treatment was prescribed. Patients in the interventional group received vitamin D (0.25 μg calcitriol) ($1,25\text{-(OH)}_2\text{D}_3$) per day orally, and the other received an organoleptically similar placebo at the same frequency for six months.

2.2. Skin prick test

To assess the specific allergen to which each patient was sensitive a skin prick test was performed for all participants. Briefly, standard glycerinated extracts (1:10 w/v) of pollen (*Amaranthus palmeri*, *Atriplex bracteosa*, *Chenopodium album*, *Salsola kali*, *Fraxinus americana*, *Ligustrum* spp., *Rumex crispus*, *Ambrosia* spp., *Artemisia* spp., *Cosmos bipinnatus*, *Helianthus annuus*, *Alnus* spp., *Quercus alba*, *Prosopis* spp., *Populus alba*, *Schinus molle*, *Cynodon dactylon*, *Lolium perenne*, *Phleum pratense*, *Holcus* spp., *Zea mays*), fungi (*Alternaria* spp., *Aspergillus niger*, *Candida albicans*, *Cephalosporium* spp., *Helminthosporium* spp., *Hormodendrum* spp., *Mucor* spp., *Penicillium notatum*, *Rhizopus* spp.), arthropods (*Dermatophagoides* spp., *Periplaneta americana*) and dog and cat antigens were used. The test included negative (sterile glycerol) and positive (histamine base 1 mg/ml) controls. The means of the largest orthogonal diameters of the papules were recorded 15 min after finger-prick administration of allergens. A positive response was recorded when the diameter of the inflamed area was 3 mm greater than that caused by the negative control.

2.3. Quantification of IgE

At the beginning and the end of the clinical trial, 8 ml of blood was obtained without anticoagulant. Serum was separated by centrifugation and stored at -20°C . IgE levels were determined using a commercial ELISA kit (Abcam® AB 108650, Mexico) according to the recommendations of the manufacturer.

2.4. Quantification of peripheral eosinophils

From anticoagulated blood, peripheral leucocytes were purified for all patients using the density gradient technique recommended for Histopaque™ (Sigma-Aldrich, St. Louis, MO). The purified cells were stained with monoclonal antibodies coupled to fluorescent markers for flow cytometry. The antibodies were specific for the antigens expressed

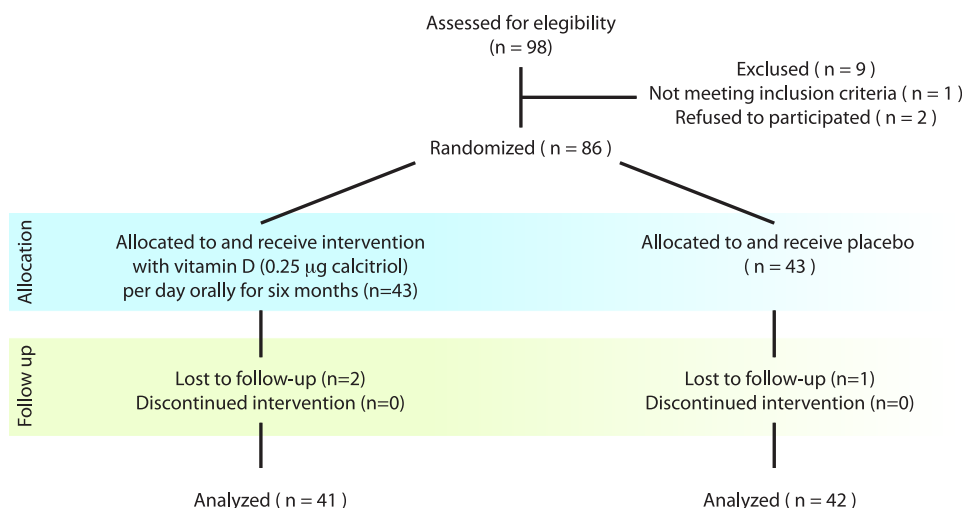


Fig. 1. Consort diagram of the study.

specifically on the surface of CD49d (Bioscience®, 9047-0499-120, Vienna, Austria) and CD69 (Abcam® AB 135933, Mexico) eosinophils.

2.5. Quantification of cytokines

The immune status of the patients was analyzed using the quantification of circulating cytokines in the serum as described above. This analysis determined the concentrations of IL-5, IL-9, IL-10, IL-13, and IFN γ (all from Peprotech™ Mexico) through ELISA. All procedures were conducted following the manufacturer's recommendations.

2.6. Bacteriology

Samples were obtained for each patient directly from the pharynx using sterile swabs. The samples were manipulated under sterile conditions to be grown in Stuart medium. The cultures were incubated in chocolate and blood agar for 24–72 h at 37 °C in the presence of 5% CO $_2$. Then, the bacterial colonies were identified according to their metabolic and morphological characteristics. Only the samples showing growth of pathogenic bacteria (*H. influenzae*, *S. aureus*, beta hemolytic *Streptococcus* spp., *S. pneumoniae*, *M. catarrhalis*, *Chlamydia pneumoniae*, *Blastocystis hominis*, and Enterobacteriaceae) were considered positive.

2.7. Cathelicidin expression

Cathelicidin LL-37 expression was evaluated using immunoblotting with the purified protein from each patient's sputum at the beginning and the end of the trial for both groups. Immunodetection was conducted using an LL-37 polyclonal rabbit antihuman antibody (AB64892 Abcam®, Mexico) and an anti-rabbit antibody conjugated to horseradish peroxidase (HRP) (Cell Signaling 7074). The immunoreactive proteins were detected by chemiluminescence with HRP substrate (Millipore WBKLS 0100).

2.8. Statistical analysis

Variables are described as means $\pm$ standard deviation or as medians (IQR) according to their distribution. Categorical variables are described as frequencies and percentages. Comparison between patients receiving calcitriol with those receiving placebo was carried out using Student's *t* test, the Mann-Whitney *U* test, χ^2 , and Fisher's exact test, as appropriate. A paired *t* test or the Wilcoxon signed rank test was used to analyze baseline values, and also after vitamin D treatment in the same group. All analyses were two sided, α was set at 5%, and *P* $\leq$.05 was considered statistically significant.

3. Results

At baseline, 86 patients were selected and randomly allocated into two groups, namely the vitamin D treated group (*n* = 43) and the control group (*n* = 43). Each patient group had similar characteristics at the beginning of the trial (Table 1).

3.1. Skin prick test

Thirty-four different allergens were tested in the patients' skin. The

results showed similar sensitivity in both groups. All patients were allergic to mite and pollen antigens. The frequencies found were *Dermatophagoides* spp. (treated 83.72%, control 81.39%), *Fraxinus americana* (treated 60.46%, control 62.79%), *Ligustrum* spp. (treated 30.23%, control 27.9%), *Populus alba* (treated 13.9%, control 13.9%), *Helianthus annuus* (treated 11.6%, control 13.9%), and *Cynodon dactylon* (treated 11.6%, control 11.6%). Notably, some patients had a positive reaction to more than one allergen, and those reactions remained unchanged at the beginning and the end of the trial. No statistically significant differences were observed between the groups (Fig. 2).

3.2. IgE levels

IgE levels before the treatment did not show statistically significant differences between the groups (treated 208 $\pm$ 98.3 IU/ml, control 204 $\pm$ 100.5 IU/ml). At six months after the start of treatment, serum IgE concentrations were significantly lower in patients who received calcitriol than in the untreated patients (treated 108.8 $\pm$ 33.8 IU/ml, control 209.8 $\pm$ 95.6 IU/ml) (Fig. 3B).

3.3. Eosinophils

The eosinophil count in peripheral blood was determined in both groups before and after the treatment. Before the treatment, no significant differences were found between groups (treated 1.3 $\pm$ 2.6 $\times 10^3/\mu$ l, control 1.31 $\pm$ 2.6 $\times 10^3/\mu$ l). After the treatment, the mean eosinophil count was statistically lower and less variable in the calcitriol-treated patients than in the control patients (treated 0.77 $\pm$ 1.3 $\times 10^3/\mu$ l, control 1.29 $\pm$ 2.5 $\times 10^3/\mu$ l) (Fig. 3A).

3.4. Quantification of cytokines

The serum levels of IL-5 and IL-9 in patients from both groups were similar at the beginning of the trial (treated group: IL-5: 33.66 $\pm$ 6.95 pg/ml; IL-9: 58.19 $\pm$ 13.94 pg/ml; control group: IL-5: 39.76 $\pm$ 13.25 pg/ml; IL-9: 58.44 $\pm$ 7.44 pg/ml). IL-13 levels were higher in the treated group (treated group: 199.7 $\pm$ 12.89 pg/ml; control group: 191.30 $\pm$ 17.66 pg/ml). However, six months later, the levels of the three cytokines were significantly lower in the calcitriol-treated group (IL-5: 7.11 $\pm$ 6.63 pg/ml, IL-9: 21.52 $\pm$ 5.88 pg/ml, IL-13: 80.07 $\pm$ 12.68 pg) than in the control group (IL-5: 40.83 $\pm$ 14.60 pg/ml, IL-9: 58.44 $\pm$ 7.44 pg/ml, IL-13: 261.52 $\pm$ 19.88 pg/ml). Furthermore, IL-10 levels were similar in both groups at the beginning of the study (treated group: 0.53 $\pm$ 0.94 pg/ml; control group: 0.48 $\pm$ 0.8 pg/ml) and IFN γ levels were lower in the treated group than in the control group (treated group: 0.53 $\pm$ 0.94 pg/ml; control group: 0.48 $\pm$ 0.8 pg/ml). Surprisingly, six months later, levels of both cytokines were significantly higher in the group treated with vitamin D (IL-10: 25.52 $\pm$ 0.43 pg/ml; IFN γ : 55.49 $\pm$ 20.32 pg/ml) than in the control group (IL-10: 0.60 $\pm$ 0.83 pg/ml, IFN γ : 2.16 $\pm$ 1.8 pg/ml) (Fig. 4).

3.5. Bacteriology

At the beginning of the study, pathogenic bacteria grew in all of the cultures from both groups of patients, which confirmed the history of recurrent respiratory tract infections in the medical records. Bacteria found in the baseline cultures were *H. influenzae*, *S. pneumoniae*, beta-hemolytic *Streptococcus* spp, *S. aureus*, and *Chlamydia pneumoniae*. Six months later, the same bacteria sampling and cultivation procedure was repeated in each group. A significant difference was found at this time. All the patients in the control group showed growth in the cultures, and the same bacteria were involved. In contrast, in the group of patients who received calcitriol, 90.3% showed no pathogenic bacterial growth and only 9.3% (4 patients) exhibited growth of *Blastocystis hominis*, a bacterium that is considered pathogenic (Table 2).

Table 1
The demographics of the trial participants.

	Treated group	Control group
<i>n</i>	43	43
Age (years) ($\bar{x} \pm$ SD)	41 $\pm$ 11	42 $\pm$ 15
Gender (female/male)	37/6	38/5

Key: *n* Number of patients in the group; $\bar{x}$ mean; SD Standard deviation.

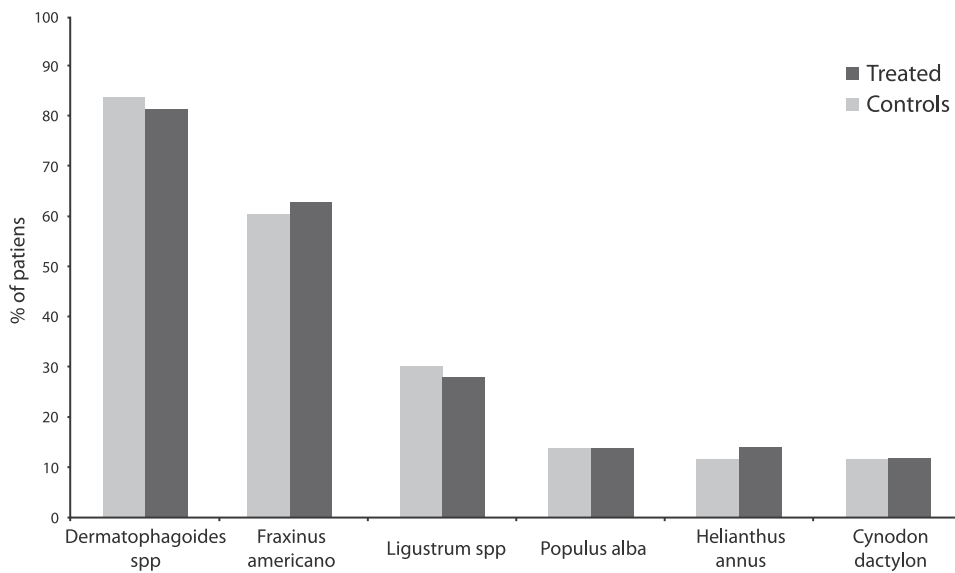


Fig. 2. Skin prick test. Percentage of patients with a positive reaction to specific allergens.

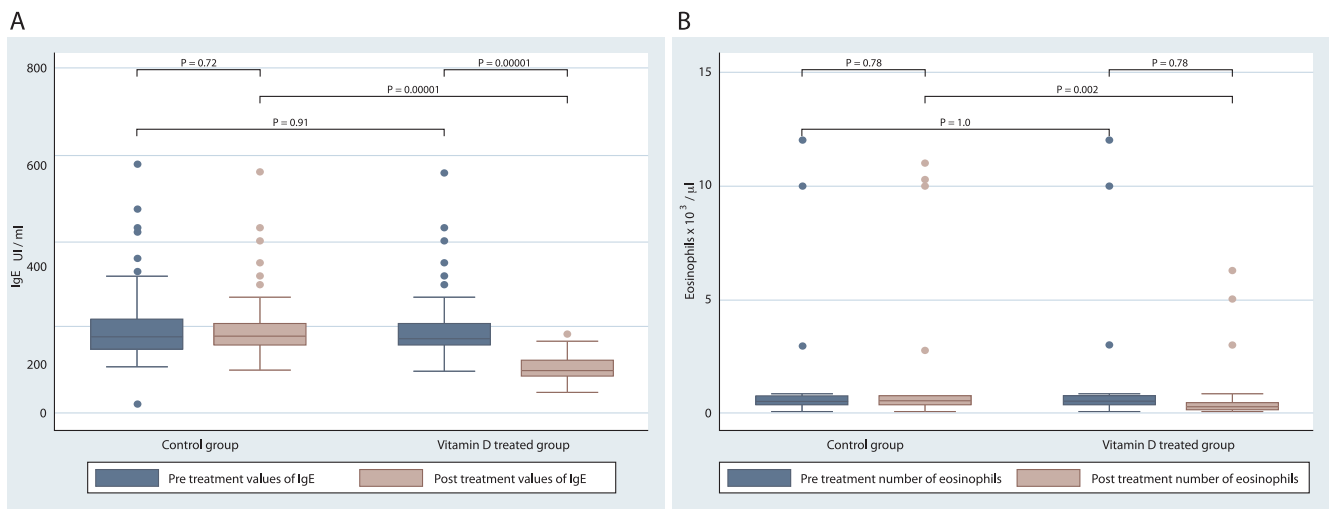


Fig. 3. IgE Levels and eosinophils number before and after vitamin D treatment. A: Serum concentrations of IgE in allergic asthmatic patients with addition of vitamin D3 treatment and the control group. B: Eosinophils in peripheral blood of asthmatic patients with addition of vitamin D treatment and the control group. The graphs show the median and standard deviation; statistical differences were determined at a value of $P \leq .05$. These data were analyzed using the U Mann Whitney and the Wilcoxon signed rank statistical tests.

3.6. Cathelicidin expression

The evaluation of cathelicidin LL-37 in the sputum of both patient groups showed that at the beginning of the trial, this antimicrobial peptide was only evident in one patient in the group that received vitamin D (2.3%) and two patients in the control group (4.5%). However, six months later, the presence of this peptide was detected in 38 patients (90%) in the calcitriol-treated group and only three patients in the control group (7%) (Table 2 and Fig. 5).

4. Discussion

Because the Mexican population is highly heterogeneous, it is necessary to establish specific protocols to prevent and treat diseases with a higher prevalence in this population [27]. Asthma is defined as a chronic inflammatory lung disease that affects approximately 300 million people worldwide and it is a polygenic disease involving complex interactions between environmental conditions and genetic factors inherent in the individual [19]. All asthma phenotypes are characterized by considerable damage to the epithelial barrier, severe inflammation of the epithelium mucus, decreased production of antimicrobial

peptides, and increased susceptibility to infection by pathogenic microorganisms in the airways [4]. For the treatment of asthma, GINA recommends combination therapy and two essential conditions, especially in patients with an allergic phenotype: (1) limitation of contact with allergens that cause allergic reactions, and (2) development of an appropriate immunotherapy to induce tolerance [20]. For the regulation of the immune response, vitamin D has been suggested as a useful tool in the treatment of asthma, and it has proven effective in reducing asthma symptoms and decreasing secondary infections by bacteria or viruses. In this work, we propose the use of vitamin D as an adjuvant in the treatment of allergic asthma. We consider essential to describe the effect of this micronutrient in the Mexican population since it has been reported that similar treatments have had variable effects in different populations [28]. A possible explanation for these variations could be the existence of polymorphic genes related to the metabolism or transport of vitamin D. For example, Ganmaa and colleagues carried out a trial in which they administered high doses of vitamin D without finding a positive effect, this finding was explained by the presence of two single nucleotide polymorphisms in 1 α hydroxylase, which is the enzyme responsible for the conversion of 25 (OH) D to the active form of 1,25-dihydroxyvitamin D [29]. This information contrast to what has

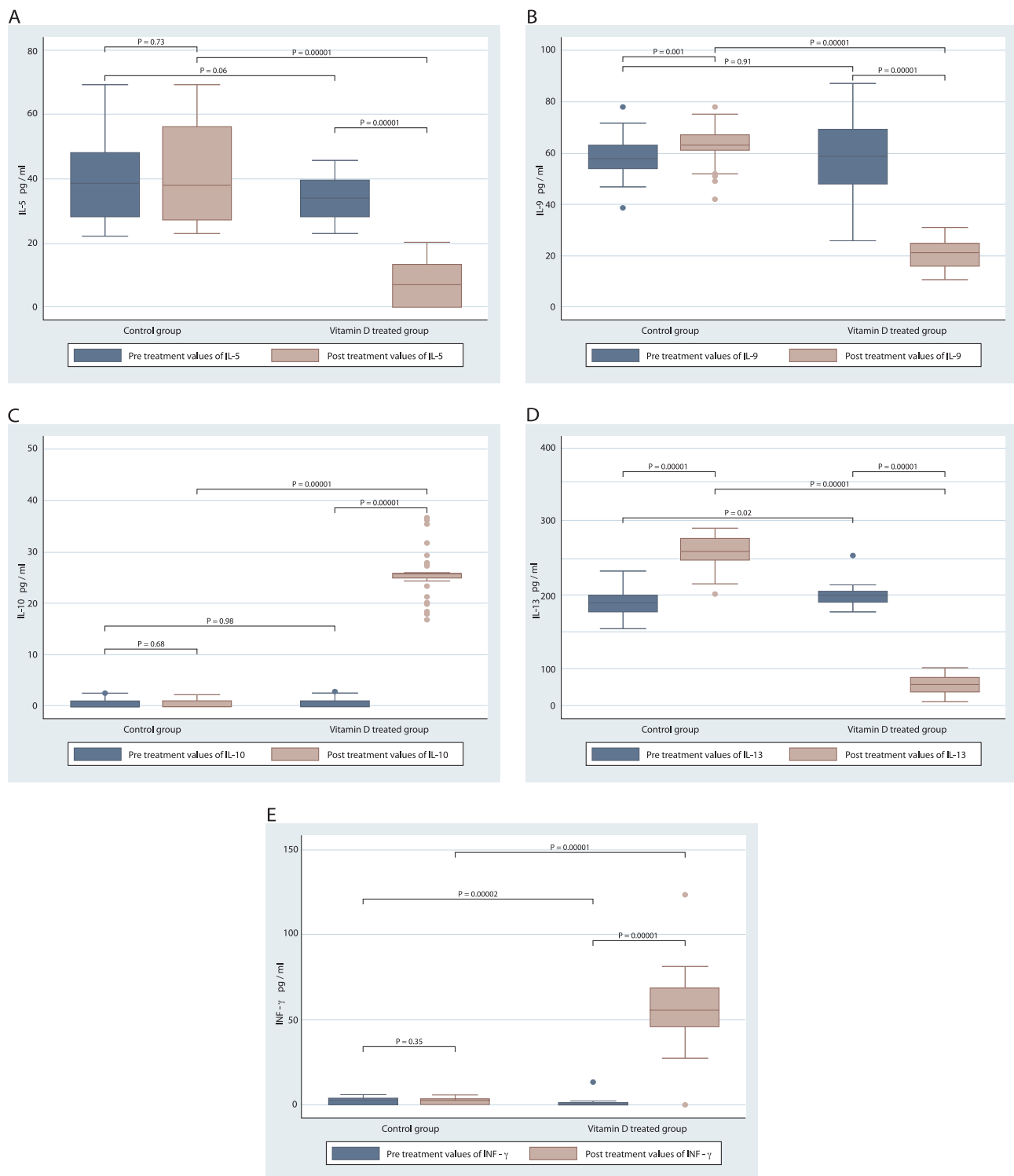


Fig. 4. Cytokine levels modification after vitamin D treatment. Serum concentration of IL-5 (A), IL-9 (B), IL-10 (C), IL-13 (D), and IFN γ (E) in patients in the vitamin D3-treated group and the control group. Levels of IL-5, IL-10, and IFN γ are shown as medians and standard deviation while IL-9 and IL-13 are shown as means and standard deviation, due to their non-parametric and parametric distributions, respectively. Analyses performed with the same group were paired. Statistical significance was determined at $P \leq .05$. The IFN γ , IL-9 and IL-13 data were analyzed using *U* Mann Whitney and the Wilcoxon signed rank statistical tests. While data from IL-5 and IL-10 were analyzed using a paired *T* test.

been reported by other authors who propose the use of this vitamin as an adjuvant in the treatment of tuberculosis [30]. Urashima et al. [21] conducted a protocol in which vitamin D was administered in the winter to children with asthma, resulting in a decrease in influenza infections compared to children with asthma who did not receive vitamin D. Another study highlighted the effect of vitamin D as a critical factor in preventing asthma exacerbations due to secondary infections

[15]. The present study focused on the phenotype of patients who have asthma with allergies and atopy, along with variable nutritional conditions, because these patients are those that are most frequently treated in the Allergy Unit of the General Hospital of Mexico. We recognize that the number of patients and the wide age range as limitations of this trial, however we consider that the results obtained are important and they can be considered for subsequent clinical studies.

Table 2
Infections and cathelicidin expression.

Variable	Patients treated with vitamin D n: 43	Patients treated with placebo n: 43	P
Patients expressing cathelicidin, at baseline, previous to experimental maneuver, n (%)	2 (5%)	1 (2%)	1.0
Patients expressing cathelicidin, after treatment with vitamin D or placebo, n (%)	37/41 [*] (90%)	3/42 ^{**} (7%)	.0001
Patients with infections prior to experimental maneuver	43 (100%) ^a	43 (100%) ^a	1.0
Patients with infections after experimental maneuver	3/41 [*] (7%) ^b	42 ^{**} (100%) ^a	.0001

Key: n Number of patients in the group.

* Two patients were lost at follow-up.

** One patient was lost at follow-up.

^a Bacteria found in pharyngeal cultures: *H. influenzae*, *S. pneumoniae*, beta-hemolytic *Streptococcus* spp., *S. aureus*, and *Chlamydia pneumoniae*.

^b Genus found in pharyngeal cultures: *Blastocystis hominis*.

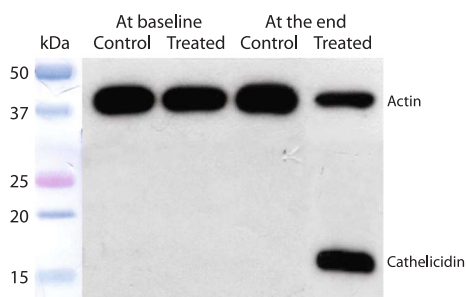


Fig. 5. Cathelicidin LL-37 induction by vitamin D treatment. Representative image of the procedure performed in each of the protocol patients. Cathelicidin LL-37 was purified from the sputum of one patient in the treated group and one in the control group, before and after the experimental maneuver.

Our objective was to analyze the effect of calcitriol supplementation in addition to the therapy recommended by GINA on the development of asthma and secondary bacterial infections in an open population without precedents in vitamin D levels.

In our trial, all patients in both groups were sensitive to one or more pollens related to the environment of Mexico City, as determined by the skin prick test. The data are consistent with previously reported data in a descriptive and observational study of the same population, in which the authors indicated that pollens are the main cause of allergy in patients living in Mexico City [31]. However, the skin prick test determined that 6 months after the start of calcitriol supplementation, patients remained sensitive to the same allergens. Recent results are mostly related to atopy markers such as IgE and eosinophil levels; both markers were evaluated in all of the patients in this study; both parameters showed a statistically significant decrease after six months in the calcitriol-treated group. Chen et al., showed that vitamin D is capable of inhibiting the transformation of B lymphocytes in plasma cells and that it indirectly decreases the production of antibodies [22]. Also, several studies reported that vitamin D inhibits the production of IgA, IgG, and IgM induced by different stimuli under *in vitro* conditions [23–32]. These prior studies notwithstanding, the explanation for the effect of vitamin D supplementation on IgE under *in vivo* conditions is complicated because the currently available information is controversial. While Heine et al., showed that vitamin D inhibits the production of IgE mediated by IL-4 under *in vitro* conditions [32], Matheu et al. reported that treatment with vitamin D increased IgE levels but decreased eosinophil levels in a model of mice pre-sensitized with albumin [33]. In this study, the decrease of IgE levels and eosinophil number was not reflected in the inhibition of sensitivity to environmental allergens; however, an evaluation for a longer treatment period may show these effects. This hypothesis is supported by the concentrations of cytokines found in these patients in response to treatment.

Eosinophil levels in this phenotype of patients are dependent on IL-5. This cytokine is known as human eosinophilopoietin, and it plays an essential role in the development of allergic asthma [34]. This study

found that the administration of calcitriol in patients with allergies induced a reduction in this cytokine; this finding is consistent with studies that used animal models of asthma, in which the authors reported that treatment and pre-treatment with 1,25(OH)(2) vitamin D(3) induces a decrease in IL-5 levels [33,35].

Nevertheless, IL-9 is considered a better severity marker than IL-5 and IL-13 in patients with allergies [36]. The present study showed that the administration of calcitriol in patients with allergic asthma decreased serum levels of IL-9. Although the effects of vitamin D supplementation for treatment of asthma patients has not been fully analyzed, there is a report that is consistent with our results. Keating et al. conducted an *ex vivo* experiment using peripheral blood mononuclear cells from patients with asthma and chronic allergy. The cells were stimulated with a specific allergen and produced low levels of IL-9 when incubated with vitamin D [37]. Various studies have highlighted the importance of IL-13 in the development of allergies. Some therapies have even focused on the reduction of this cytokine because its decrease is associated with tissue remodeling and clinical improvement [38]. In our study, the IL-13 reduction was induced by the addition of vitamin D to the treatment of asthma patients. This finding is significant because it is the first report on the effect of vitamin D on this cytokine under *in vivo* conditions.

Our results showed that patients with asthma and bacterial infections who were treated with vitamin D had higher levels of IFN γ than patients who did not receive vitamin D. These data contrast with several reports that showed that vitamin D administration in patients with different pathologies reduces serum levels of IFN γ [39–41]. Although the effect of adding vitamin D supplementation to the treatment of patients with allergic asthma has rarely been explored, the existing data indicate that immune regulation is different in allergic diseases than in other diseases. Ozkara et al. conducted a study that measured IFN γ in the serum of patients with polyposis and allergic rhinitis, and in patients with polyposis unrelated to allergy; both groups received vitamin D. Surprisingly, the authors found that only the patients with polyposis and allergic rhinitis had increased levels of IFN γ [42]. In addition, the activation of the enzyme CYP11A1 in CD8⁺ T cells by Th2 cytokines has been reported to cause increased IL-13 and decreased IFN γ , but vitamin D inhibits this change and induces increased production of IFN γ by these cells [43]. Moreover, a beneficial and close relationship between vitamin D and IFN γ has been reported. Adams et al. demonstrated a feedback mechanism whereby high levels of IFN γ promoted hydroxylation of the 25OHD substrate, leading to the synthesis of intracellular 1,25(OH)2D and thus higher levels of the active form of the vitamin [44]. On the other hand, it has been reported that IL-18 induces IFN gamma expression [45] and although it has been proposed that the serum concentrations of IL-18 and IL-18 binding protein play a significant role in the development of asthma [46]. The scope of this proposal remains uncertain because of IL-18 is a cytokine produced by the activation of inflammasome [47], a molecular mechanism associated with the phenotype of neutrophilic asthma [48] as opposed to allergic asthmatic patients of this protocol. However, all of this

information suggests that phagocytic cells activated by IFN γ have a greater capacity to kill invading microorganisms.

In this trial, we have demonstrated that the administration of vitamin D in patients with allergic asthma induces increased serum levels of IL-10. This finding agrees with previous reports showing similar effects on IL-10 in animal models [49] and humans [50]. Furthermore, the increase in serum levels of IL-10 is associated with an anti-inflammatory effect on eosinophilic [51] and neutrophilic [52] asthma. Although high levels of IL-10 were not associated with a reduction of the inflammatory reaction in the skin prick test in this study, this possibility should be explored in future analyses using longer periods of treatment with vitamin D in the same type of patients.

In this work, the most relevant clinical effect of treatment with vitamin D in asthma patients was the reduction of bacterial respiratory infections. Thus, it was not necessary to use antibiotics during the study period. This effect can be explained by an increase in the microbicidal capacity of the cells of the immune system. The mechanisms by which effector cells of the immune system remove germs can be divided into two types: oxidative and non-oxidative. Non-oxidative mechanisms involve antimicrobial peptides, which are particularly important for innate immunity. Cathelicidin LL-37 is known for being inducible by vitamin D [53]. We explored the expression of cathelicidin LL-37 in allergic asthma patients receiving vitamin D supplementation and in a group of patients with the same pathology without this supplementation. Expression of this peptide was significantly higher in patients who received vitamin D, and this change was negatively correlated with bacterial infections, which were the main cause of exacerbations in these patients. Given this statistical correlation, we propose that one of the causes for the reduction of respiratory infections may be the increase in mechanisms of innate immunity.

The scientific literature has multiple reports of the relationship between vitamin D and various diseases; however, almost all of them have attempted to correlate serum levels of vitamin D with susceptibility or resistance to diseases. Few works have explored the effect of vitamin D supplementation on the treatment of diseases. In our trial, we focused on a specific phenotype of allergic asthma with a particular feature (presence of bacterial infections) as a key factor in the occurrence of exacerbations. The present study showed an immunoregulatory effect of vitamin D with a clear clinical outcome, which was the control of bacterial infections due to an increase in an essential mechanism of innate immunity: the antimicrobial peptide LL-37. However, this effect on immune regulation and allergic status should be evaluated using prolonged treatment periods. Vitamin D is far from being a cure for the allergic condition of patients because atopy involves the genetic factors of each individual; however, vitamin D proved to be an excellent adjuvant that favors a decrease in exacerbations and restores homeostasis in the organism.

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Trial registration

DI/14/309/04/003.

Conflict of interest

None.

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