Resetting the balance of Cytokine by Vitamin D3 in Asthmatic Patients

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Abstract: Bronchial asthma is an allergic disorder characterized by excessive hyperactive nature of the airways, that depends on many cytokines as interleukin 4 and 5 (IL 4 & 5) which are responsible for the allergic inflammatory response. One of the strategies in the management of bronchial asthma is the induction of synthesis of interleukin 10. Interleukin 10 has an inhibitory effect on the synthesis of the T-helper 2 cytokines (Th2). Th2 cells are required for the development of airway eosinophilia and the secretion of interleukines 4, 5 and 13. Also, it has been demonstrated that vitamin D3 has a role in overcoming the receptor down regulation induced by glucocorticoids. The aim of this study is to assess the therapeutic effect of vitamin D3 in patients with steroid dependent asthma, as an additional option in treatment. A case control study was conducted among two groups of patients. The first group comprised 40 patients who received treatment of vitamin D3 for one month (40 patients); While the second group was the control group (40 patients) who did not receive treatment. Each participant was subjected to comprehensive assessment, skin prick test, and the level of serum calcium. IL2, 1L4 and IL 10 was measured. The results revealed that there was a statistically significant increase in the IL10 level post treatment in group I, while IL4 and IL2 decreased significantly after treatment. It can be concluded from the current study that active vitamin D3 is of benefit in patients with moderate to severe asthma, as implicated from the cytokine profile and clinical response criteria and pulmonary function tests. However this new complementary form of treatment needs to be confirmed by further studies.

1. Introduction

Inflammation plays an integral part of the clinical response in bronchial asthma. This allergic inflammatory response is a result of a complex interaction between various inflammatory cells, their mediators on the airway epithelium and smooth muscle. Inflammation in the airways leads to airway narrowing secondary to airway remodeling performed by the Th2 cells, eosinophils, mast cells, and other leucocytes leading to airway thickening **(Lambrecht, 2006)**. Asthmatic symptoms include wheezing, cough and sputum production resultant from mucus hyper secretion **(Renauld, 2001)**.

Th2 are subsets from CD4 T cells that induce their effector mechanisms through the eosinophils and mast cells and induce B cells to produce IgE. These cells are required for the development of airway eosinophilia and secrete IL4, IL5, and IL13 **(Janeway et al., 2005)**. Th2 cells were thought to be the major inducers of effector mechanisms that led to the development of asthma. IL4 is responsible for initial priming of the Th2 cells, and thus necessary for the initial differentiation **(Herrick and Bottom ly, 2003)**. When natural killer cells become activated, they respond with a similar cytokine profile to that of the Th2 cells

and produce IL4 and IL13 **(Meyer et al., 2006)**.

However the immunological role of IL I0 was demonstrated through its inhibitory effect on cytokine secretion from allergic specific Th2 cells **(Lee et al., 2002)**. ILI0 also has direct inhibitory effects on antigen presenting cells (APCs) and T cells: it also modulates eosinophil accumulation in airways by possibly inhibiting eosinophil production in bone marrow. Those with asthma have decreased expression of IL10 demonstrating its important role in regulating allergic diseases **(Janeway et al., 2005)**.

Since asthma and other allergic diseases are a result of Th2 dominated responses, it was initially thought that resetting the cytokine balance to induce Thl would counterbalance Th2 activity. While this method is efficient in suppressing the eosinophilic airway inflammation, a Thl response does not have a desired effect on reducing allergic responses **(Tournoy et al., 2006)**. Resetting the cytokine balance to regulatory T cells rather than to Thl cells may result in a decrease in asthmatic symptoms **(Akbari et al., 2003)**.

Vitamin D is essential for active calcium absorption, longitudinal bone growth, activity of osteoblasts and osteoclasts **(Lips, 2006)**. The

active metabolite 1, 25 (OH)2D has an antiproliferative effect and down regulates inflammatory markers. Also it is essential for the paracrine regulation of cell differentiation and function. Moreover, it has pleiotropic effects through its receptor and response elements of many genes, and on the other side rapid non genomic effects through membrane receptor and second messengers **(Lips, 2006).**

Growing evidence indicates that 1,25 (OH) 2D3 is a modulator of immune system function, consistent with its capacity to control cellular differentiation **(Mathieu et al., 2001).** Also There was also a positive correlation with allergy subtypes such as prevalence of rashes, sneezing, and sinus infections with low vitamin D (Frieri M, Valluri A.2011). Moreover it regulates the T cell activity both directly and indirectly through modulation of antigen presenting cell function **(Adorini et al., 2003).** Treatment reduces expression of the cytokine IL 12, whose signaling is critical for Th1 maturation. Also, 1,25(OH)2D3 directly represses the transcription of genes encoding Th 1 associated cytokines such as IL-2 and interferon gamma **(Takeuchi et al., 2001). Litonjua 2012** stated that of the fat-soluble vitamins, vitamin D holds great promise as an agent for primary and secondary prevention of disease.

2. Methods
Study design:
A case control study

Sample: 80 patients were included in this study aging front 18- 55 years. The patients were selected from those attending at the outpatient clinic of Ain Shams university hospital. They were subdivided into two groups:

Group I:
Forty patients received 0.5 ug oral vitamin D3 daily for one month.

Group II:
Forty patients who did not receive such treatment.

Patients were subjected to full history taking. forms of corticosteroid whether oral or inhaled according to the severity of asthma. Clinical examination was done, skin prick testing, pulmonary function test (FEV1/ FVC ratio), Serum calcium, IL-10, IL-2, and IL-4 (ELISA) before and after treatment. Patient who were smokers and who were not on oral or inhaled corticosteroids were

excluded from the study. Classification of asthma severity was done according to GINA Classification 2005.

Skin prick testing was done to detect the specific IgE antibodies to inhalant allergens and food allergens. The test was performed on normal skin, positive and negative controls were excluded, and the use of extracts for testing at appropriate concentrations.

Spirometry was done to evaluate degree of air way obstruction, and to evaluate the forced expiratory volume in the first second (FEV1).The device used is called the spirometer which measures the amount of air exhaled in a 6 second period duration after deep inspiration. However, obstruction is defined as a ratio less than 70% of the FEV I to the forced vital capacity.

Laboratory measurement: IL2, IL4 and ILI0 were measured using the kit of **(Accucyte Sciences INC, Maryland USA)** based on a competitive enzyme immunoassay technique. Expected normal values for IL-2 up to 15pg/ml, while IL4 up to 35pg/ml, and IL-10 expected values up to 16.0 pg/ml.

Statistical analysis: Statistical Package for social Science program, version 12 was used for analysis of the data as follows: Qualitative data was presented in the form of number and percentage, while quantitative data was presented in the form of mean ±SD and range. Unpaired t-test was used to compare two groups as regard quantitative variables in parametric data; Paired t-test was used to compare quantitative variables in the same group. Wilcoxn test was used for non-parametric data assessment. While, the Chi-square test was used to compare qualitative variables between two groups. Spearman correlation coefficient test was used to rank variables against each other's either positively or inversely.

3. Results
Studying the gender difference among the two groups revealed that group I comprised of 19 females and 21 males. In group II there were 15 females and 25 males. No statistically significant difference between the studied groups regarding the gender by using the chi-square test. The mean ages of the participants in the two groups were 38.8+/-8 and 36+/- 4 respectively. There was no statistically significant difference between the studied groups regarding the disease duration by using the unpaired t-test; whereby group I was 20.2+/-9 years and group 11 was I 8.7+/- 7.5 years of bronchial asthma.

Table (1) Severity Score after Treatment in Group I

Severity score	Before	After	X2	P
Mild	0	12(30%)	8	<0.05
Moderate	8 (20%)	20 (50%)		S
Severe	32(80%)	8 (20%)		

This table shows that there is a statistically significant improvement in the severity score after treatment with vitamin D3.

Table (2) Severity Score after Treatment in Group II

Severity score	Before	After	X2	P
Mild	0	4 (10%)		>0.05
Moderate	8 (20%)	8 (20%)	1.3	NS
Severe	32 (80%)	28(70%)		

This table shows that no statistically significant difference could be detected in severity score after treatment by usual treatment.

Table (3) Changes in the Serum Calcium after Treatment

Serum Calcium	Group I N=40	Group 11 N=40	T	P
Before	9.3+/- 0.4	9.2+/-0.4	0.3	>0.05 NS
After	9.7+1-0.4	9+/- 0.4	2.3	<0.05 S

This table shows that group I had a higher serum calcium level compared to group II after treatment which was statistically significant. While, there was no significant difference before treatment.

Table (4) Changes in serum IL-10 after treatment among studied groups

IL-10	Before	After	% of change	T	P
Group 1	12.5+1-2	23.5+/-5.2	83%	8	<0.01 HS
Group 11	12.1+/-1.9	13.3+/-1.8	8%	2	<0.05 S

This table shows that IL-10 was increased post-treatment in comparison to before treatment with statistically highly significant difference in group I; while a significant change in group II by using paired t-test.

Table (5) Changes in serum IL-4 after treatment among studied groups.

IL-4	Before	I After	% of change	T	P
Group 1	48+/-9	32+/-6	33%	9	<0.01 HS
Group 11	39.5+/- 5.5	37.8+/-5	5%	2.5	<0.05 S

This table shows that IL-4 was decreased post treatment in comparison to before treatment with highly statistically significant difference among group I, and significant difference among group II using paired t-test

Table (6) Changes in serum IL-2 after treatment in group I.

Variables	Mean	+/- SD	Z	P
Before	47.3	24	2.5	<0.05
After	43	28		S

There is a significant decrease in the level of IL2 after treatment by using Wilcoxn sign test.

Table (7) Correlation between severity score after treatment versus other variables among group I.

Variables	Severity score	P	Significance -
Age	0.19	>0.05	NS
Disease Duration	0.12	>0.05	NS
S. Ca	0.14	>0.05	NS
1L-10	-0.47	<0.05	S
IL-4	0.39	<0.05	S

This table shows statistically positive correlation between severity score versus IL-4 and inverse correlation versus IL-10 by using Spearman correlation test.

When comparing both groups regarding the severity score according to GINA Guidelines 2005, there was no statistically significant difference by using the chi-square test. However, changes in the severity score after treatment with vitamin D revealed a statistically significant improvement when using the chi-square test.

4. Discussion

Studying the laboratory occurring in the

cytokine profile in asthmatic patients secondary to the administration of vitamin D3 is considered an important point of research work. Bronchial asthma has acquired some unresponsiveness to ordinary therapy. Therefore this work was done to offer an additional therapy to overcome this problem by oral intake of vitamin D3. Vitamin D3 acts as a modulator of the immune system, by its ability to regulate the proliferation and differentiation of a wide variety of cells.

This study was conducted on 80 patients, all of which were atopic and non-smoker asthmatics on oral or inhaled corticosteroids with different degrees of severity. These patients were selected from the outpatient allergy clinic in Ain Shams university hospitals. The patients were subjected to full history, examination and skin prick test, as well as pulmonary function test. From the eighty patients, forty received active vitamin D3 0.5ug daily for one month and IL-2, IL-4, IL-10 and serum calcium were done before and after intake of active vitamin D3. The other group received only their usual asthma treatment but without active vitamin D3 and IL-2, IL-4, IL- 10 and serum calcium were measured before and after treatment.

In the current study it was found that a significant decrease in the severity score after treatment in group I in comparison to group II with a p value <0.05. This was in agreement with **Hawrylowicz et al., 2005** who stated that when vitamin D was added to dexamethasone upon the culture medium the T-cell defect was reversed. In addition, there was a highly significant increase in interleukin 10 after active vitamin D3 treatment in group I P<0.01. While there was a significant decline in 1L-2 and IL-4.

Xystrakis et al., 2006 also reported that oral administration of active vitamin D3 in severe steroid resistant asthma enhanced IL-10 response to corticosteroid therapy by enhancing its synthesis. However, this contribution to the clinical efficacy of dexamethasone was given only for one week.

However our findings might be in contrast to the findings of **Mai et al 2012** who found that The serum 25(OH)D level was not associated with incident asthma in women, regardless of allergy status. Low vitamin D status was not significantly associated with incident asthma in most adults, but it may have increased risk among men without allergy.

Treatment of bronchial asthma aims at influencing the Th 1 /Th2 balance. Asthma is a result of a Th2 dominant response, so resetting the cytokine balance to induce Th1 would counterbalance Th2 activity. Th1 response can be induced by IL-12, INF gamma and infectious agents **(Tournoy et al., 2006).** While this method is effective in suppressing eosinophilic inflammation, it does not attenuate the allergic response. Therefore, resetting the cytokine balance to regulatory T cells may result in a decrease in asthmatic symptoms **(Akbar et al., 2003).**

IL-4 carries out many functions as the regulation isotype switching in B cells to IgE, chemokine production and activation of mast cells and eosinophils. In addition, 1L-4 is essential for initial priming of the Th2 cells and thus necessary for their initial differentiation **(Herrick and Buttonly, 2003).** Moreover, it was found that this cytokine was increased in patients with steroid resistant asthma, resulting in local resistance to the anti-inflammatory action of glucocorticoids **(Irusen et al., 2002).**

Patients with steroid resistant asthma have statistically significant reduction in the capacity of their CD-4 T cells to respond to dexamethasone for inductions of IL-10 synthesis **(Hawrylowiez et al., 2002). Xystrakis and colleagues** stated in 2006, that oral administration of vitamin D3 enhanced the IL-10 production from the regulatory T cells; which subsequently inhibits the cytokine production from allergen specific Th2 cells. Moreover, vitamin D3 enhances the IL-10 response to dexamethasone, thus overcoming the resistance on the down regulated glucocorticoid receptors induced by dexamethasone.

This functional significance is also supported by **Franchimont and colleagues in 2001,** who, observed increased sensitivity of monocytes to dexamethasone induced by IL-10. Vitamin D3 was observed to rescue the expression of corticosteroid receptors (CR) among the CD4 T cells. However, vitamin D3 has no direct effect on CR expression on the CD4 T cells, but to its ability to promote IL-10 synthesis by glucorticoids **(Chikanza, 2002) (Keating et al 2014).**

Steroid resistant asthma occurs due to several factors including several abnormalities in either glucocrticoid receptor number, as well as other factors as decreased IL-10 secretion by T-regulatory cells, and P38 Mitogen-activated protein MAP kinase activity **(Adcock et al., 2003**). **Spahn and colleagues in 2007** found a modulation of the vitamin D receptor in the bronchial smooth muscles, expressing its gene regulatory effect in the pathogenesis of bronchial asthma in asthmatic patients.

The results of the current study should be confirmed by other studies with a larger number of participants, to allow detection if early administration of vitamin D3 in asthmatic patients and other allergic conditions. Also, to search for further investigations, or other mechanisms offered by the use of active vitamin D3 as an adjuvant therapy in asthmatic patients. It can be concluded from the current study, that active vitamin D3 has a potential effect of on

improving the symptom score, and resetting the cytokine balance when compared to usual forms of therapy. So that vitamin D3 could have an immunomodulatory role in asthmatic patients, by increasing the receptor sensitivity to steroids in asthmatic patients. Therefore, this addition may provide an adjuvant therapy in treatment of moderate to severe bronchial asthma especially in patients with low vitamin D intake and who are kept indoors with no adequate sun light exposure.

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