Original article

Effects of vitamin D and the antimicrobial peptide in asthma

Background: *Vitamin D modulates a variety of processes and regulatory systems including host defense, inflammation, and immunity. A connection between Vitamin D status and asthma has been considered. Vitamin D mediates innate immunity, particularly through enhanced expression of the human cathelicidin antimicrobial peptide (LL-37). Increased levels of high sensitive-C reactive protein (Hs-CRP) were found to be significantly associated with respiratory function impairment.* ***Objective:*** *to evaluate the relation between serum vitamin D, Hs-CRP and LL-37 levels and asthma.* ***Study design:*** *Thirty children (15 males and 15 females) with proven diagnosis of asthma (ages ranged from 3-13 years) were studied; they attended the pediatric department of Zagazig University Hospital in the year 2011. In addition, 30 age and sex matched apparently healthy children served as a control group. All children were subjected to history taking, clinical examination, laboratory investigations (CBC, CRP, ESR), determination of serum level of 25-hydroxyvitamin D (25 OHD) and plasma LL-37.* ***Results:*** *The study revealed a highly significant decrease in 25 OHD, LL-37 and a highly significant increase in Hs-CRP in children with asthma than in control group. There were a highly significant positive correlation between vitamin D and LL37 in patients' and control groups and a significant negative correlation between both 25OHD and LL37 and Hs-CRP in patients' group. High WBC count (specially neutrophils and lymphocytes), Hs-CRP level and low levels of hemoglobin, 25OHD and LL 37 in patient group were considered risk factors of asthma.* ***Conclusion:*** *Inappropriate concentration of vitamin D decreases the ability of the immune system to defend against infection through lowering LL-37 and elevated Hs-CRP which leads to occurrence and precipitation of asthma.*

Keywords: Vitamin D- LL-37- asthma

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INTRODUCTION

In the last years, it has been recognized that in addition to the classical role of vitamin D in calcium and bone homeostasis, Vitamin D modulates a variety of processes and regulatory systems including host defense, inflammation, immunity, and repair. Several lung diseases, all inflammatory in nature, may be related to activities of Vitamin D including asthma and COPD[1]. Vitamin D deficiency has been blamed as one causes of increased asthma prevalence in the last decades[2]**.** The underlying mechanisms how Vitamin D modulates the pathogenesis of asthma is not clear. Vitamin D may modulate the function of various immune cells such as B- and T-lymphocytes[3]. Interestingly, application of Vitamin D is potentially capable to overcome the poor glucocorticoid responsiveness in severe asthmatics by upregulation of IL-10 production from CD4+ T cell[4]**.**

Vitamin D–mediated innate immunity, particularly through enhanced expression of the human cathelicidin antimicrobial peptide (hCAP-18), is important in host defenses against respiratory tract pathogens[5]**.**

Increased levels of high sensitivite-C reactive protien (Hs-CRP) were found to be significantly associated with respiratory function impairment and bronchial hyperresponsiveness (BHR)[6,7], suggesting that systemic inflammation may be associated with respiratory impairment and that Hs-CRP might be a sensitive marker for severe asthma.

Aim of the work

To evaluate the relation between serum vitamin D, Hs-CRP and LL-37 levels and asthma.

METHODS

This case control study included 30 children (15 male and 15 female) with proven diagnosis of asthma. Their ages ranged from 3-13 years (X±SD: 7.37±3.10 years) and were attending Pediatric Clinic, Zagazig University Hospital during the year 2011. They were selected randomly one every three cases and were divided into 4 subgroups according to GINA guidelines[8].

1. Mild intermittent (12 children, 6 males & 6 females).
2. Mild persistent (10 children, 5 males & 5 females).
3. Moderate persistent (6 children, 2 males & 4 females).
4. Severe persistent (2 male children).

Thirty healthy children (16 males, 14 females) of ages ranging from 2-13 years with a mean of (7.10 ± 2.94 y) were studied as a control group. Ethical approval was obtained from the local research ethics committee and parents of all children gave an informed written consent prior to the study.

All children were subjected to the following:

1. History taking including age, sex, residence, socioeconomic level, cough, chest tightness.
2. Clinical examination including a transverse crease on the nose, conjunctival congestion and inflammation, tachypnea, subcostal, intercostal retraction, barrel-shaped chest, prolonged expiration, rhonchi, wheezes, tachycardia.
3. Complete blood count (CBC) done on Sysmex SF3000 cell counter (Seimens, Germany).
4. High sensitive-CRP determination. Serum concentrations of high-sensitive CRP were measured using an automated nephelometric method (BN ProSpec, Seimens, Germany); it is a closed system analyzer with its specially manufactured kits from the same company. Hs-CRP levels ≥6 mg/L were considered as increased[9].
5. Measurement of serum 25-OH vitamin D: 2 ml venous blood sample was obtained, centrifuged and serum was separated and stored at -20ºC until assayed. Serum levels of 25-OH vitamin D were measured after extraction using the Immunodiagnostic Enzyme-Immuno-Assay (EIA) developed by Immundiagnostic, Bensheim and Biomedica, Wien, Australia[10]. Catalog number 02082005 25 OH vit D6.DOC. The cut off level of vitamin D was 35 nmol/l.
6. Measurement of human plasma LL-37: 2 ml venous blood samples were collected on EDTA, centrifuged and plasma was separated. The plasma stored at -70ºC in polypropylene tubes until assay. The human LL-37 was measured using solid-phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle using a commercial human LL-37 ELISA kit, HK321 Hycult Biotech, Fronststraat 2a,5405PB Uden, the Netherlands[11]. The cut off level of LL 37 was 20 ng/ml.

Statistical analysis:

Data were presented as mean±standard deviation (X±SD) or percentage (%). The means of two groups were compared using student "t" test. Chi square (χ^2) was used to find the association between row and column variables. Linear correlation and regression were used to test the correlation between the measured parameters and the studied groups. Odds ratio was used to quantify the risk. Cut off values were calculated from the ROC curve as mean±2SD of control. Data were tabulated and statistically analyzed with the statistical package for Social Sciences (SPPS), version 10 software. P-values less than 0.05 were considered significant[12].

RESULTS

Analysis of demographic characteristics of the studied groups revealed that there were nonsignificant differences between patients and controls as regard age, sex, weight and residence (table 1). Table 2 shows the clinical and radiological presentations of the patient group. Cough and rhonchi were the most presenting symptoms and signs respectively. Table 3 shows the laboratory data of studied groups. No statistical significance was obtained between the two groups as regard white blood cell count, hemoglobin level and platelet count.

Our study showed a highly significant decrease in 25OHD, LL-37 and a highly significant increase in Hs-CRP in children with asthma than in control group (table 4). The lowest levels of 25OHD, LL-37 and highest level of Hs-CRP were found in patients with moderate persistent and severe persistent asthma (table 5). There was a highly significant positive correlation between 25OHD and LL37 in patients, control groups and a significant negative correlation between both 25OHD and LL37 and Hs-CRP in patients group (table 6). High WBC count (specially neutrophils and lymphocytes) and Hs-CRP level and low levels of hemoglobin, 25OHD, LL 37 in patients' group were considered risk factors of asthma (table 7).

Table 1. Demographic characteristics of the studied groups.

Variables	Patients (n = 30)	Controls (n = 30)	t / χ^2	p
Age (years) X±SD	7.37 ± 3.10	7.10 ± 2.94	0.342	0.734
Weight	23.03 ± 7.50	22.13 ± 6.33	0.502	0.618
Sex (No, %)				
Male	15 (50%)	16 (53.3%)	0.067	0.796
Female	15 (50%)	14 (46.7%)		
Residence				
Rural	22 (73.3%)	20 (66.7%)	0.317	0.573
Urban	8 (26.7%)	10 (33.3%)		

Table 2. Clinical and radiological presentations of the patients' group.

	No	%
Symptoms:		
1.Cough	28	93.3
2.Wheezes	15	50
3.Chest tightness	8	26.7
4. Nonspecific symptoms (which includes a persistent cough with colds, and/or chest rattling).	6	20
Signs:		
1.Tachypnea	5	16.7
2.Prolonged expiration	10	33.3
3.Tachycardia	5	16.7
4.Rhonchi	21	70
5.Barrel-shaped chest	3	10
6.Chest x-ray	1	3.3
7. Other signs (which includes, a transverse crease on the nose, conjunctival congestion and inflammation and subcostal and intercostal retractions).	7	23.3

Table 3. Laboratory data of the studied groups.

Variables	Patients (n = 30) X±SD (Range)	Controls (n = 30) X±SD (Range)	t	p
White Blood cell count ($x10^3/mm^3$)	9.45 ± 3.72 (5 – 17)	7.95 ± 1.79 (5 – 11)	1.988	0.053
Hemoglobin (g/dL)	11.50 ± 1.21 (8.9 – 13)	11.31 ± 0.90 (10 – 13)	0.700	0.487
Platelet count ($x10^3/mm^3$)	313.00 ± 62.54 (160 – 450)	332.00 ± 47.15 (250 – 420)	1.329	0.189

Table 4. 25-OH Vitamin D, LL-37 and Hs-CRP levels among the studied groups.

Variables	Patients n = (30)	Controls n =(30)	t	p
25-OH Vitamin D (nmol/l)	62.97 ± 24.50	181.87 ± 90.38	6.955	< 0.001*
LL-37 (ng/ml)	5.80 ± 1.89	11.59 ± 4.45	6.571	< 0.001*
Hs-CRP(mg/l)	6.57 ± 1.76	1.21 ± 0.38	16.290	< 0.001*

Table 5. Relation between 25-OH vitamin D, LL-37 and Hs-CRP levels and the severity of asthma.

Variables	Mild intermittent and mild persistent asthma (n = 22)	Moderate and severe persistent asthma (n = 8)	t	p
25-OH Vitamin D (nmol/l)	69.36 ± 25.56	45.38 ± 6.70	4.038	< 0.001*
LL-37(ng/ml)	6.47 ± 1.82	4.69 ± 0.47	4.222	< 0.001*
Hs-CRP (mg/l)	6.10 ± 1.09	7.85 ± 2.59	1.846	0.103

Table 6. Correlations among the studied laboratory parameters.

		LL-37		Hs-CRP	
		r	p	r	p
25-OH Vitamin D	**Control**	0.465	0.010*	0.253	0.178
	Patients	0.526	0.003*	- 0.335	0.070
LL-37	**Control**			0.372	0.043*
	Patients			- 0.138	0.466

Table 7: Risk factors for the development of asthma

	Patients (n = 30)	Control (n = 30)	OR (95% CI)
Residence			
Rural	22 (73.3%)	20 (66.7%)	1.375
Urban	8 (26.7%)	10 (33.3%)	(0.453 – 4.170)
WBCs count			
Normal	24 (80%)	30 (100%)	2.250
Abnormal (increased)	6 (20%)	0 (0%)	(1.670 – 3.032)*
Hemoglobin level			
Normal	19 (63.3%)	26 (86.7%)	3.763
Abnormal (decreased)	11 (36.7%)	4 (13.3%)	(1.038 – 13.646)*
Platelet count			
Normal	30 (100%)	30 (100%)	-
Abnormal	0 (0%)	0 (0%)	
Vitamin D (nmol/L)			
< 75	21 (70%)	3 (10%)	21
> 75	9 (30%)	27 (90%)	(5.047 – 87.373)*
LL-37 (ng/ml)			
< 10	30 (100%)	9 (30%)	0.231
> 10	0 (0%)	21 (70%)	(0.130 – 0.409)*
Hs-CRP (mg/dl)			
< 6	13 (43.3%)	30 (100%)	3.308
> 6	17 (56.7%)	0 (0%)	(2.101 – 5.209)*

DISCUSSION

Vitamin D insufficiency is widespread and is associated with increased incidence of respiratory tract infections and asthma. This association may be particularly important for individuals with asthma, for whom respiratory tract infections often trigger asthma exacerbation and may increase the frequency, severity, and duration of lower respiratory tract symptoms[13]. Our study showed that there were nonsignificant differences between patients and controls regarding age, sex and associates residence. Kosti, et al,[14] declared that children living in athens had higher likelihood of asthma compared with children living in rural areas this may be due to the higher age of patients of this study.

The commonest complaint in our study was cough (93.3%). As stated by Castro-Rodríguez, et al,[15] cough may be the only symptom of asthma. Also 50% of our patients had wheezes which is one of the most common symptoms of asthma[16]. In this study there were nonsignificant differences between patients and controls regarding white blood cell count, hemoglobin level and platelets count.

In our series, asthmatic patients had significantly lower 25OHD levels than controls. A connection between vitamin D deficiency and

asthma has been considered since many years. Low levels of serum vitamin D is associated with impaired pulmonary function, increased incidence of inflammatory and infectious diseases. The exact mechanisms underlying these data appear to impact on the function of inflammatory and structural cells, including dendritic cells, lymphocytes, monocytes, and epithelial cells[17]. Ginde, et al,[18] added that the association between serum Vitamin D level and upper respiratory infection was much stronger among individuals with asthma compared with those without asthma. This increased risk of respiratory infections may contribute to incident wheezing illness in children and cause asthma exacerbations. There may be a cause-and-effect relationship between vitamin D deficiency and the increased incidence of asthma because vitamin D deficiency is associated with increased airway hyperresponsiveness, lower pulmonary functions, worse asthma control, and possibly steroid resistance[19]. In addition lung epithelial cells express high baseline levels of 1α-hydroxylase. This allows the conversion of inactive calcidiol to active calcitriol locally within the lung. Calcitriol has been shown to inhibit the synthesis and release of certain cytokines, such as platelet-derived growth factor, and matrix metalloproteinases, from bronchial smooth muscle cells, thereby leading to decreased lung inflammation and smooth muscle cell proliferation. Also Vitamin D increases synthesis of interleukin 10 by CD4_CD25_Foxp3_ T-regulatory cells and dendritic cells, while concurrently inhibiting dendritic cell activation by down regulating expression of co-stimulatory molecules CD40 and CD80/86[20].

Our results showed significant correlation between low 25 OHD level and the severity of asthma. These were in agreement with Brehm, et al,[21] who found that children with lower vitamin D levels were significantly more likely to have been hospitalized for asthma, tended to have airways with increased hyperreactivity and were likely to have used more inhaled corticosteroids, all signifying higher asthma severity. Vitamin D deficiency increases the risk of severe asthma exacerbation and the need for emergency department evaluation or hospitalization; it may be related to asthma severity in several ways. First, vitamin D influences the immune system through its effects on helper T cell type 1 and type 2, and regulatory T cells. Second, current vitamin D intake may influence lung function in patients with asthma, similar to its potential effects in non-asthmatics. Third, vitamin D stimulation has been shown to influence micro array gene expression signatures in bronchial smooth muscle cells. Fourth, polymorphisms in the gene encoding the vitamin D receptor (VDR) have been associated with asthma phenotypes, none of these polymorphisms resulted in an amino acid change in the translated protein, so the mechanism of increased asthma susceptibility is related to regulation of VDR expression[22].

In our study LL-37 levels were significantly lower in patients than controls and there was a significant correlation between low LL-37 levels and the severity of asthma. Vitamin D–mediated innate immunity, particularly through enhanced expression of the human cathelicidin antimicrobial peptide (LL-37), is important in host defenses against respiratory tract pathogens which precipitate asthma[18]. In addition, Xiao et al,[23] showed that patients with asthma show a dramatic decrease of LL-37.

We found a highly significant positive correlation between LL-37 and 25OHD. Vitamin D stimulate the secretion of natural antibiotics known as antimicrobial peptides (LL-37), this gives vitamin D the potential to combat a range of infections[24]. It regulates the production of antimicrobial peptides such as LL-37 in cultured macrophages[25]. Also Jeng, et al,[26] found a positive association between vitamin D status and plasma LL-37, which suggests that systemic LL-37 levels may be regulated by vitamin D status. In addition Camargo, et al,[20] found an association between lower blood vitamin D levels and increased respiratory infection, increased wheezing, and decreased LL-37 levels. So the human cathelicidin (LL-37) appears to have a particularly important role in the vitamin D–mediated mechanism against infection.

In our study Hs-CRP levels were significantly higher in patients than controls. Jousilahti, et al,[27] stated that in asthma, beside the importance of airway inflammation, systemic inflammation may exist based on the increased serum Hs-CRP levels. Increased levels of Hs-CRP were found to be significantly associated with respiratory function impairment and bronchial hyperresponsiveness. Hs-CRP is a sensitive marker for inflammation, infection and tissue damage, and contributes to the host defense against infection by activating the complement pathway. A positive association has been reported between raised Hs-CRP levels, asthma, respiratory impairment and bronchial hyper-reactivity[28].

Our study showed a significant negative correlation between both 25OHD and LL37 and Hs-CRP in the patients group. Ford,[29] found a positive correlation between raised CRP levels and asthma,

respiratory impairment and bronchial hyperresponsiveness. Whereas Devaraj, et al,[30] reported a correlation of peripheral blood CRP levels with severity, extent, and progression of inflammatory pathology.

The present study showed that high WBC count, low hemoglobin level, low vitamin D level, low LL-37 level and high Hs-CRP level were risk factors of asthma. Low vitamin D level was the most important risk factor.

In conclusion, inappropriate concentration of vitamin D decreases the ability of the immune system to defend against infection through lowering LL-37 and elevated Hs-CRP which leads to occurrence and precipitation of asthma. So, we recommend appropriate vitamin D supplementation and sun exposure to decrease occurrence of asthma and its severity and performing further studies to define the correlation between vitamin D, LL-37, Hs-CRP and asthma, also study the role of vitamin D as a therapeutic agent in the treatment of asthma.

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