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Research Article

Assessment of Vitamin D and Iron Levels in Childhood Asthma

Hanan M Hamed[1*], Gamal Abdel Naser Yamamah[1], Nabil Abd El-Aziz Ibrahim[2], Nevine Elsaid Mohammed Elhelaly[2], Eman A Wadalla[3], Mahmoud Mohamed Shahat[1]

[1]Pediatric Department, National Research Centre), El-Buhouth str., Dokki, Cairo, Egypt
[2]New Children Hospital, Cairo University), El-Buhouth str., Dokki, Cairo, Egypt
[3]Clinical Pathology Department, National Research Centre), El-Buhouth str., Dokki, Cairo, Egypt

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ABSTRACT

Associations between an unhealthy diet and asthma are reported. A recent attention has focused on the possible relationship between vitamin D and iron and the presence of asthma. The aim: To assess the levels of serum Vitamin-D and iron in children complaining of asthma to demonstrate their possible effect on asthma and pulmonary function. Methods: A cross sectional, case control study was designed. It included sixty patients with partly controlled asthma and uncontrolled asthma according to GINA criteria,2010 were recruited from Asthma Clinic, New Children's Hospital, Cairo University from 20 September 2013 until 30 Mai 2014.Thirty healthy age and sex matched are included as control. All children were subjected to full history and clinical examination. Serum vitamin D and iron were measured. pulmonary function was done. Results: 33.3% and 25% of control and asthmatics patients have insufficient vitamin D levels (20-30 ng/ml). The frequency of asthmatics patients with iron deficiency is significantly higher than control (46.7% Vs 16.7%) (p= 0.004). In uncontrolled group, vitamin D was correlated positively with absolute eosinophilic count (p= 0.002 &r = 0.5) and negatively with FEV_1(p = 0.01&r = - 0.5). Conclusion: High prevalence of vitamin D insufficiency in both asthmatic and healthy children. Vitamin D may play a role in asthma pathogenesis. Serum iron of asthmatics was lower than the healthy group. Recommendation Further prospective studies on large scale aiming the use of an inexpensive intervention in a substantial number of asthmatic children.

Keywords: vitamin D, vitamin D deficiency, serum iron, asthma.

INTRODUCTION

Asthma is a common and potentially serious chronic disease that imposes a substantial burden on patients, their families and the community. It causes respiratory symptoms, limitation of activity and flares ups that sometimes require urgent health care and may be fatal[1]. Its main physiological feature is episodic airway obstruction characterized by expiratory airflow limitation and the dominant pathological feature is airway inflammation sometimes associated with airway structural changes[2]. Approximately 300 million people worldwide currently have asthma, with estimates suggesting that asthma prevalence increases globally by 50% every decade. Prevalence is higher (>10%) in developed countries and, however the rates are increasing in developing regions as they become more westernized[3]. Associations between an unhealthy diet, overweight and the presence of asthma are reported[4]. Moreover, a recent attention has focused on the possible relationship between vitamin D deficiency (VDD) and the pathogenesis of allergic diseases[5,6]. whereas there is a lack of consistent data addressing the topic of VD supplementation in the prevention of food allergies[7]. Several epidemiological studies have reported significant associations between VDD and increased risk of childhood wheezing and asthma exacerbation[8]. Some studies supports that Vitamin D has a role in asthma pathogenesis through increasing Th2 responses, airway smooth muscle cells expression & functions and increasing respiratory infections. However, the precise role of VD in the immune system and in allergic respiratory diseases still remains to be clearly defined[9,10]. Anemic children were 5.75 times more susceptible to asthmatic attacks when compared with non-anemic children[11]. Iron deficiency exerts adverse effects on immune response and alters the metabolism of growth of pathogens. It has already been reported that low hemoglobin impairs tissue oxygenation and acts as an independent risk factor for developing lower respiratory tract infections and bronchial asthma in the children[12]. So we aimed to assess the levels of serum Vitamin-D and Iron in children complaining of asthma both partly controlled and uncontrolled and to demonstrate their correlation with the pulmonary function.

PATIENTS AND METHODS

A cross sectional, case control study was designed. It included sixty patients of both sexes; their age ranged between 6 – 16 years. They were categorized into two groups the 1st included 30 patients with partly controlled asthma; the 2nd group included 30 patients with uncontrolled asthma according to GINA criteria,2010[2]. They were recruited from Asthma Clinic, New Children's

**Author for Correspondence: hmhamed63@yahoo.com*

Hospital, Cairo University from 20 September 2013 until 30 Mai 2014. Any patient received iron or vitamin D therapy during the last 6 months was excluded from the study. Thirty healthy children age and sex- matched were included in the study as a control group, they were recruited from the follow-up and surgical clinics of the hospital. Written informed consents were taken from the guardians of all children. The study was approved by the ethical committee of the National Research Centre. All children were subjected to full history with stress on (history of vitamin D and iron supplementation during last 6 months before the study and detailed history about dietary intake of vitamin D and iron), history of medication. physical examination was done with emphasis on symptoms and signs of asthma & criteria of control. Sign of vitamin D deficiency. Wight and height for each subject were measured using standard techniques. Body mass index was calculated using standard equation. Values of weight, height and BMI were calculated as percentile of norm for age and sex. Pulmonary function tests were performed using a spirometer (Fukuda Denshi, Spirosift SP5000). Proper instructions on how to perform the forced expiratory maneuver must be given to each patients, and the highest value of minimally three technically acceptable maneuvers is taken. Spirometric parameters include most of the lung volumes, capacities and flow rates as: Vital Capacity (VC), Forced Vital Capacity (FVC), Forced Expiratory Volume in the First Second (FEV_1) and Peak Expiratory Flow Rate (PEFR). Five ml of venous blood were withdrawn; 2 mills were collected into the lavender vacutainer tubes that contain EDTA to assess HB level, Total Iron Binding Capacity (TLC) and Absolute Eosinophilic count. Three mills were centrifuged to get a serum samples to assess vitamin D and iron. Blood films were prepared from EDTA anticoagulated blood then Hb concentration is estimated by Colorimetric techniques or spectrophotometer, based on light intensity principle The cell counting component counts the numbers and types of different cells within the blood; the remaining part of sample was stained with Leishman stain; blood films were examined, differential leukocytic count was done manually and Eosinophil were counted in 100 white blood cells and expressed as percentage. Serum iron was analyzed using OLYMPUS Coulter method using TPTZ [2, 4, 6-Tri-(2-pyridyl)-5-triazine] as the chromogen. In an acidic medium using serum sample. Dynamic Range: The Iron procedure is linear from 10 to 1000 µg/dL, With Reference Values: Child 60 - 120 µg/dL[13]. Expected values may vary with age, sex, diet and geographical location. Total Iron Binding Capacity (TIBC) was analyzed using OLYMPUS automated analyzers after precipitations. Serum plasma samples, free from hemolysis, are the recommended specimens. The Total Iron B Child: 200-380 µg/dL[14]. Expected values may vary with age, sex, diet and geographical location. Total iron bindinding Capacity assay is linear from 55 – 450 µg/dL. Vitamin D (25-Hydroxy Vitamin D): was analyzed using Human 25-hydroxyvitamin D (25-(OH) D) Elisa Kits manufactured at Glory Science Co., Ltd. 2400 Veterans Blvd. Suite 16 - 101, Del Rio, TX 78840, USA. The reference range of vitamin D is 30-74 ng/ml[15]. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. The range of the kit is: 0.5 ng/mL-> 200 ng/mL with Sensitivity: 0.22 ng/mL

Statistical Analysis

Data was analyzed using SPSS win statistical package version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using student t-test. ANOVA test was done to compare between the three groups in our study. Post-hoc test was done to examine the significant difference between groups if present. Pearson correlation coefficient was used to examine the relationship between two quantitative variables. P-value < 0.05 was considered significant.

RESULTS

Sixty patients, 27 males and 33 females with mean age 8.71 ± 2.67 years were included in the study. The demographic data of the patients and controls were shown in table 1. The percentile height of asthmatic children was significantly lower than healthy controls (p = 0.035) (table 1). Complete blood count showed that, asthmatic groups (partly controlled & uncontrolled) had higher mean values of WBCs than that of healthy control with a significant difference (p= 0.004 & 0.003 respectively). Asthmatic groups (partly controlled & uncontrolled) showed a higher mean values of absolute eosinophilic count (AEC) than the healthy group with a significant difference (p= 0.001 & 0.001 respectively) (table 2). Table 2 showed no significant difference in the mean values of serum vitamin D level between the studied groups. Serum iron of partly controlled & uncontrolled groups were significantly lower than that of healthy group (p=0.01& 0.009 respectively). While TIBC is in normal range and no difference between them. The

Table 1: demographic data of asthmatics and healthy controls

Item	Asthmatic children n=60 mean± S.D.	Healthy controls n=30 mean± S.D.	p-value
Age (years)	8.71 ± 2.67	8.90 ± 2.25	0.722
Gender (Females/Male)	33/27	18/12	0.923
Weight (centile)	48.15 ± 26.74	53 ± 21.28	0.389
Height (centile)	35.72 ± 27.33	49.00 ± 20.06	0.035
BMI (centile)	53.22 ± 29.76	50.83 ± 29.69	0.721

Table 2: comparing the study population characteristics between healthy controls and the cases groups (partly controlled and uncontrolled

Item	Healthy control	P value *	Partially controlled	P value * *	uncontrolled	P value * * *
HB(gm/dl)	11.86 ± 1.61	0.495	12.10 ± 0.978	0.531	12.44± 1.00	0.10
WBCs (cells/mm3)	5819 ± 1471	0.004	7573 ± 2771	0.974	7444 ± 2509	0.003
AEC (cells/mm^3)	208 ± 119	0.001	423 ± 319	0.598	499 ± 401	0.001
Vitamin D (ng/ml)	58.8± 53.1	0.13	43.2±19.2	0.1	44.6±33.1	0.2
Serum iron (mcg/dl)	77.6±33.9	0.01	56.6±28.8	0.8	55.2±30	0.009
TIBC (mcg/dl)	336.1±107.9	0.3	323±133.7	0.1	357.1±149	0.7

HB (Hemoglobin), WBCs (White Blood Cells), AEC (Absolute Eosinophilic Count), TIBC (Total iron binding capacity)

*comparing healthy controls with partly controlled asthmatics.

**comparing partly controlled with uncontrolled asthmatics.

***comparing healthy controls with uncontrolled asthmatics.

Table 3: Pulmonary function tests for the studied groups

Item	Healthy controls (n=30)	p-value *	Partly controlled (n=30)	p-value **	Uncontrolled (n=30)	p-value ***
	mean± S.D		mean± S.D		mean± S.D	
FVC%	91.33 ± 7.95	0.29	85.47 ± 12.71	0.102	89.10 ± 8.61	0.85
FEV1%	87.20 ± 7.02	0.001	75.23 ± 10.08	0.827	76.83 ± 13.51	0.001
FEF 25-75%	72.57 ± 12.02	0.064	59.17 ± 24.28	0.523	65.57± 28.57	0.46
PEFR%	88.90 ± 6.72	0.001	66.17 ± 10.79	0.869	67.80 ± 17.52	0.001

*comparing healthy controls with partly controlled asthmatics.

**comparing partly controlled with uncontrolled asthmatics.

***comparing healthy controls with uncontrolled asthmatics.

frequency of vitamin D insufficiency (20-30 ng/ml) as % of the total in each group were shown in figure (1). We found that 33.3%, 23.3%, 26.7% of control, partially controlled and uncontrolled asthmatics patients had insufficient vitamin D levels with no significant difference. No one of our studied groups had vitamin deficiency < 20 ng/ml. The frequency of decreased serum iron levels < 60 µg/dL as % of the total of each group were shown in figure (1). The frequency of iron deficiency in partially controlled and uncontrolled asthmatics patients were significantly higher than control (46.7%, 45.1 Vs 16.7%) (p= 0.004, p= 0.004) respectively. Pulmonary function values of both partly controlled group and uncontrolled group were significantly lower than those of healthy control regarding FEV1& PEFR, p-values were (0.001& 0.001) and (0.001& 0.001) respectively (table 3). There was a significant negative correlation between AEC and FEV1 in all asthmatics patients (p=0.03&r=-0.24). In uncontrolled group, vitamin D was correlated positively with AEC

(p= 0.002 &r = 0.5) (figure 2) and negatively with FEV_1(p = 0.01&r = - 0.5) (figure 3). On other hand there was no correlation between serum iron and pulmonary functions

DISCUSSION

Asthma is a major public health problem[16]. Whereas the prevalence of asthma in industrialized nations may have recently reached a relative plateau[17]. there have been recent and pronounced increments in asthma prevalence in many non-industrialized countries[16]. The causes of this global "asthma epidemic" remain largely unidentified but are likely multifactorial and may include changes in dietary patters (e.g. decreased consumption of fruits and vegetables, and increased intake of refined grains, red meats and saturated fats[18]. In the present work, anthropometric measures were assessed through the study groups; the mean value of percentile height of asthmatic children was significantly lower than that value of healthy controls (p = 0.035), as most of uncontrolled asthmatics were on regular inhaled corticosteroids which is in agreement with other[19,20]. Our results are in contrast to *(Skoner et al*., 2011)[21] who found that no significant difference between patients who received corticosteroids and placebo. In the current study, absolute eosinophilic count (AEC) was significantly higher in both asthmatic groups than that of healthy control group. This result is in concur with *Schleich et al. 201*[22]. who found that patients exhibiting eosinophilic inflammation both in blood and sputum had more severe asthma and poorer asthma

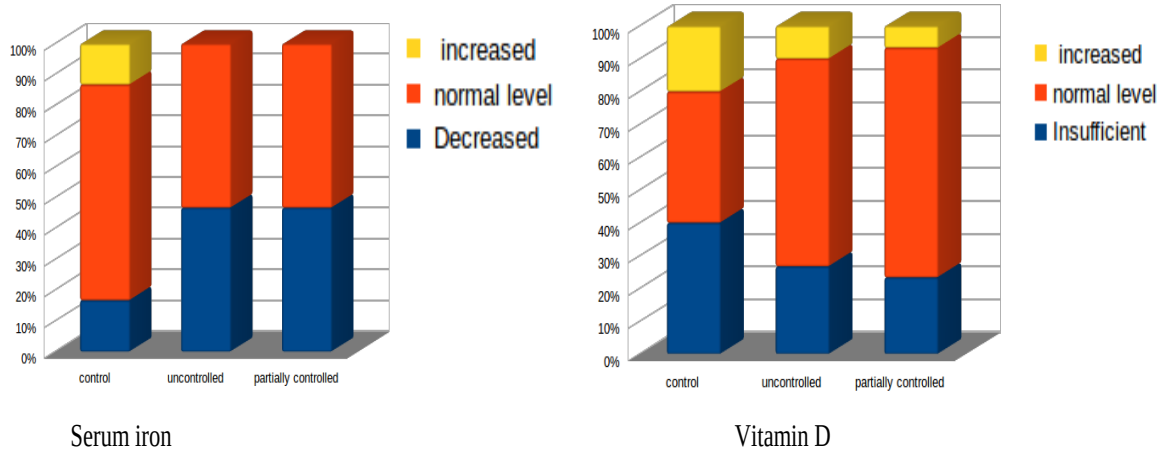

Figure 1: The frequency of vitamin D and iron levels as % of the total in each group

control than non- eosinophilic asthmatics. They showed that isolated sputum eosinophilic inflammation is associated with impaired airway caliber and increased airway hyper responsiveness compared with non-eosinophilic asthmatics, while those combining both systemic and eosinophilic had further functional impairment, which is likely to partly contribute to worse asthma control and quality of life. We found a significant negative correlation between AEC and FEV1 in the whole asthmatic cases (partly controlled and uncontrolled) which is in agreement with (Nadif et al. 2009)[23]. who found that patients with high blood eosinophilia (>250 cells/ mm^3) had lower FEV1 values and poor asthma control than those with normal blood eosinophil counts. It is thought that micronutrients influence the immune system and may play a major role in the development of asthma and in the progression of other allergic diseases therefore, the search for causes of the increasing prevalence of asthma among children, especially in developing countries, may be linked too dietary factors[4]. In the present work serum vitamin D (25-(OH) D) levels were insufficient in 25% and 33.3% of the asthmatic subjects and healthy control groups respectively. The mean absolute serum VD values were lower in asthmatic group with no significant difference between them and control subjects. Our result is in agreement with *Sohila et al., 2011*[24]. who found no significant difference between asthmatics and control regarding VD insufficiency and deficiency. Vitamin D deficiency is highly prevalent even in sun-replete areas of the world. Some possible explanations include behavioral factors as sunscreen use, indoors, and clothing coverage, and intrinsic factors such as skin melanin content, ethnicity (dark-skinned person), and decreased cutaneous production of vitamin D3[25]. *Barman et al., 2015* found that respiratory allergy did not differ between patients and controls regarding serum 25-OHD levels or calculated vitamin D intake[26]. Many studies found that a higher prevalence of VD deficiency among asthmatics group compared to non-asthmatics[27,28]. However, others found that lower 25-hydroxy vitamin D levels were associated with a higher asthma inflammatory and immunological markers[29]. Regarding the relation between vitamin D status and pulmonary function tests, we found a significant negative correlation between serum Vitamin D and FEV_1 in the asthmatic children, that are in acceptance with *Gergen et al., 2013*[30]. who found that increasing 25(OH) D concentrations were associated with an increase in number of hospitalizations, a decrease in FEV1/FVC, and an increase in the number of positive aeroallergen skin tests. *Kolokotroni et al. 2015*, reported that, there was a negative correlation between vitamin D levels and the number of reported asthma severity indicators as emergency room visits, hospital admission and frequency of asthma attacks[27]. Others, did not identify an association between FEV1% and with 25-OH vitamin D level in their study[31]. On other hand, Uysalol et al. 2013, concluded that the vitamin D level decreased the severity and the frequency of asthma. A positive and significant correlation of vitamin D and FEV1, the improvement in FEV1 occurred after vitamin D supplementation[28]. Many studies found no significant relationship was shown between serum levels of 25-hydroxyvitamin D and eosinophilic count[29,24]. However on our study, we found postive correlation between them, that is go parrele with the studies supports that Vitamin D has role in asthma pathogenesis. However, the precise role of VD in the pathogenesis of asthma is still debated and needs further assessment. Published studies had conflicting results and most of them are observational[9]. An epidemiological study showed the association between the levels of dietary iron and the prevalence of allergic diseases is still lacking[32]. In our work, 16.67% of the healthy control and 46.7% of the asthmatic case group had a decreased serum iron level with significant difference between them, that was parallel with Vlasić et al. 2009[33]. who found that asthmatics patients had a statistically significantly lower iron concentration than the control group. It is supported by others who found that children with high cord concentrations of iron were less likely than those with low concentrations to wheeze in infancy[34].

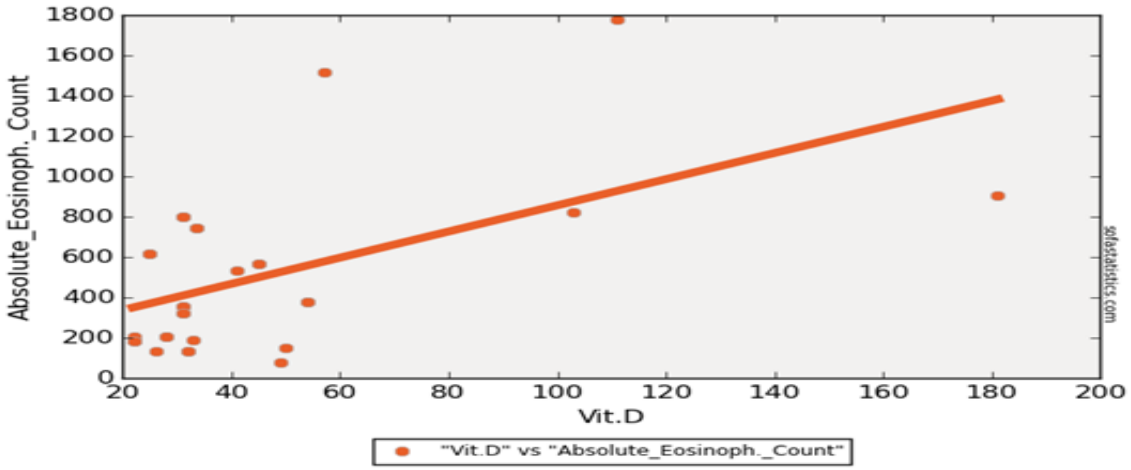

Figure 2: The correlation between vitamin D and absolute eosinophilic counts.

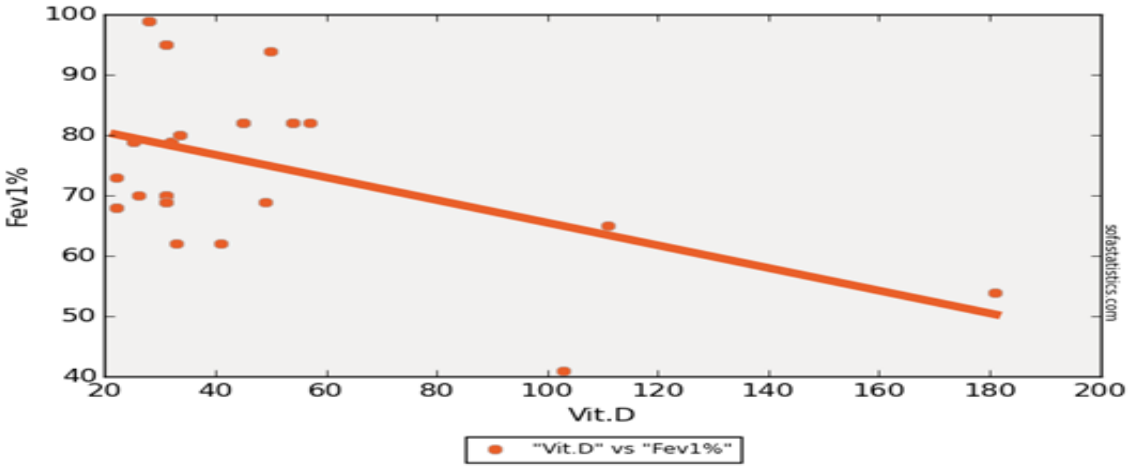

Figure 3: The correlation between vitamin D and FEV1

Emily et al., 2015 found that higher iron stores were inversely associated with asthma. They recommended,
the study of the role of iron status in asthma morbidity, study the effect of iron on mast cells and eosinophils, which are known to play key roles in the asthmatic response and trials of iron supplementation for asthma prevention[35]. Our results came in contrast to Urushidate et al.,2010 who found that no significant differences were seen in serum iron concentrations between bronchial asthma and control[36]. In contrast to our result, Narula et al. 2007reported a significant increase in plasma iron levels in asthmatic subjects as compared to controls; an increased plasma iron levels in asthmatics may contribute to aggravate lipid peroxidation as part of oxidative stress[37]. This study has a limitation, we did not follow up patients after iron and /or vitamin D supplementation and determine the severity and the frequency of asthma.

CONCLUSION

High prevalence of vitamin D insufficiency in both asthmatic children and healthy children, without significant difference between them. However, vitamin D may play a role in asthma pathogenesis, there were negative correlation of vitamin D with FEV_1 and positive correlation with absolute eosinophilic count in asthmatic groups. Serum iron of asthmatics groups were lower than the healthy group without correlation with pulmonary function.

Recommendation

Further prospective studies on large scale examining the potential mechanisms of vitamin D and serum iron in asthma pathophysiology aimed the using or not of them in a substantial number of asthmatic children.

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