**A Study of the Relationship between Serum 25-Hydroxyvitamin D Levels and Severity of Bronchial Asthma in Children**

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**Background:** vitamin D has an important role in innate immunity, including the prevention of respiratory tract infections. Vitamin D exerts many of its effects through contact with vitamin D receptors, which have been found in a variety of cells, including lung cells and many cells of the immune system

**Objective:** TOevaluate of the role of vitamin D in bronchial asthma.

**Subjects and Methods:** The study was conducted on 120 children (60 asthmatics and 60 healthy controls) during the period of from March 2012 to September 2012. Patients were attending the Pediatric Chest Clinic, Children’s Hospital, Faculty of Medicine, Ain Shams University. Serum 25 hydroxyvitamin D level was done by ELISA in both patients and controls.

**Results:** The mean value of serum 25-OH vitamin D of patients (26.98±6.59 ng/ ml) was significantly lower than that of controls (42.67±7.26 ng/ ml) (p value < 0.01). Vitamin D level decreases with increased asthma severity.

**Conclusion:** The mean serum 25-OH vitamin D of patients group was significantly lower than control group. Lower levels of serum vitamin D are associated with increased asthma severity.

**Keywords:** Serum 25 OH-vitamin D, vitamin D deficiency, bronchial asthma, Children.

**INTRODUCTION:**

 Vitamin D is a steroid hormone essential for calcium homeostasis and maintenance of bone health. ***(1)*** There are also other benefits of vitamin D that have been reported. ***(2)*** Autocrine and paracrine effects of vitamin D are becoming increasingly recognized, and may also play a role in critical illness**. *(3)***

 Vitamin D is produced in the skin through a photolytic reaction of 7-dehydrocholesterol induced by ultraviolet B radiation (290–315 nm). It also occurs naturally in foods. The metabolite formed in the skin and the vitamin D absorbed in the gut must be hydroxylated in the liver to form 25-hydroxyvitamin D [25(OH)D] and then hydroxylated in the kidney to form 1,25-dihydroxyvitamin D [1,25(OH)2D]. ***(4)***  After these transformations, vitamin D is a biologically active substance, a hormone that is chemically akin to steroid hormones. The main function of vitamin D in the body is to regulate calcium and phosphorous homeostasis, a process essential for bone mineralization**. *(5)*** Vitamin D deficiency is known to lead to secondary hyperparathyroidism, which causes rickets in children and osteomalacia and osteoporosis in adults. ***(6)***

 **Ginde et al., *(7)*** suggested the role for vitamin D in innate immunity, including the prevention of respiratory tract infections**. Liu et al.,** ***(8)*** showed that the action of vitamin D was a key link between Toll-like receptor (TLR) activation and antibacterial responses in innate immunity. They showed a dose-dependent up-regulation of one known antimicrobial peptide (cathelicidin) in human monocytes. Clarification of therole of vitamin D in relation to infections, such as acute respiratory tract infections, deserves a high priority**. *(9)*** Furthermore, vitamin D is known to play a role in the human antimicrobial response ***(8,10)*** and pulmonary function**. *(11)***

Vitamin D deficiency has been shown to be a risk factor for several chronic diseases**. *(5)*** Vitamin D exerts many of its effects through contact with vitamin D receptors, which have been found in a variety of cells, including lung cells and many cells of the immune system. ***(12)***

**AIM OF THE STUDY:**

Toassess serum level of vitamin D in a sample of Egyptian asthmatic children and determination of the impact of vitamin D deficiency on disease severity.

**SUBJECTS AND METHODS**

 This study is a case control study done on 120 children, sixty asthmatic patients aged from 6 to 12 years, who were attending the Pediatric Chest Clinic, Children’s Hospital, Faculty of Medicine, Ain Shams University during the period from March 2012 to September 2012 and sixty healthy children, age and sex matched served as a control group. Signed approval consents were taken from parents.

A- Patients group: it comprised 60 asthmatic children, the patients group was sub- classified according to asthma severity into 3 subgroups **(GINA, 2011) *(13)***:

Subgroup I: patients with mild persistent asthma.

Subgroup II: patients with moderate persistent asthma.

Subgroup III: patients with severe persistent asthma.

B- Control group: it comprised 60 healthy children age and sex matched with group A.

**INCLUSION CRITERIA:**

1. Children with persistent asthma (mild persistent, moderate persistent and severe persistent asthma).
2. Asthmatic children aged from 6 to 12 years.

**EXCLUSION CRITERIA**:

1. Children with mild intermittent asthma.
2. Children suffering from co-existing chronic disease (e.g. D.M., Rheumatic fever, etc).

**All cases were subjected to the following:**

A- Complete history taking andthorough clinical examination laying stress on chest examination.

 B- Spirometry ***(Lemanske and Busse, 2010) (13)***.

C- Laboratory investigations:

1-Complete blood count (CBC) using coulter counter (T660 Coultronics, France) with total and differential leucocytic count, eosinophil count.

2- Serum calcium, phosphorus and alkaline phosphatase using photometric method using kits purchased from Human Gmb H-65205 Wiesbaden-Germany according to the manufacture’s instruction.

3-Serum 25 OH-vitamin D level was measured using ELISA by 25-OH vitamin D EIA Kit (EPBE).

 From each child of the present study, three milliliters of blood were collected. The sample was centrifuged and the serum was stored in -20 °c till the time of analysis. The serum collected was used to determine the serum level of 25 OH-vitamin D, serum level of calcium, phosphorus and alkaline phosphatase.

**STATISTICAL ANALYSIS**

All studied statistical methods were performed using SPSS. All numeric variables were expressed as Mean ± SD.

**RESULTS**

This study was conducted in 120 children, 60 patients suffering from bronchial asthma, with a mean age of **8.60±2.10** years, 39 (65%) males and 21 (35%) females. 60 children (36 males and 24 females) with a mean age **8.70±2.24 years** were enrolled as a control group.

There was no significant difference between patient and control groups regarding age, sex,parental smoking and the presence of a history of rickets.

There was a positive history of bronchial asthma, allergic rhinitis and lower frequency of sun exposure in patients as compared to control group (tab.1).

The height was significantly lower in patients than controls (fig.1) and BMI was significantly higher in patients than controls (fig.2).

**Table (1): Demographic data of the studied groups:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sig.** | **Control group** **(60)** | **Patient group** **(60)** |  |
| **P-value** | **t** | **Mean±SD** | **Mean±SD** |  |
| **0.836****NS** | **-0.208** | **8.70±2.24** | **8.60±2.10** | **Age in years** |
| **P-value** | **X**  | **N (%)** | **N (%)** |  |
| **0.643****NS** | **0.215** | **36 (60%)****24 (40%)** | **39 (65%)****21 (35%)** | **Gender** **Males** **Females**  |
| **0.000****HS** | **19.289** | **2 (0.3%)****58 (99.7%)** | **30 (50%)****30 (50%)** | **Family history of bronchial asthma****+ve****-ve** |
| **0.370****NS** | **0.803** | **24 (40%)****36 (60%)** | **30 (50%)****30 (50%)** | **Parental smoking****+ve****-ve** |
| **0.002****HS** | **9.204** | **4 (0.6%)****56 (99.4%)** | **22 (36.7%)****38 (63.3%)** | **History of Allergic rhinitis** **+ve****-ve**  |
| **0.000****HS** | **13.78** | **54 (90%)****6 (10%)** | **30 (50%)****30 (50%)** | **Sun exposure** **+ve****-ve**  |
| **0.671****NS** | **0.179** | **10 (16.7%)****50 (83.3%** | **8 (13.3%)****52 (92.7%)** | **History of rickets** **+ve****-ve** |

**Figure (1): Height percentiles in patients and control groups.**

Figure (1) shows that height centiles were lower in patients than controls**.**

**Figure (2): Body mass index (BMI) in patients and Controls.**

Figure (2) shows that BMI centiles were higher in patients than controls.

There were significantly low serum levels of vitamin D in patients than controls was detected (table 2 ).

There were significantly high levels of blood absolute eosinophilic count was detected in patients than controls (table 3).

There were significantly high levels of serum alkaline phosphatase in patients than controls (table 4 ).

Serum level of calcium was normal in patients and controls with no significant difference (table 5 ).

**Table (2): Comparison between Cases and controls as regards serum vitamin D levels**:

|  |  |  |  |
| --- | --- | --- | --- |
| **Sig.** | **Control group** **(60)** | **Patient group** **(60)** |  |
| **P-value** | **t** | **Mean±SD** | **Mean±SD** |
| **0.000****HS** | **9.950** | **42.67±7.26** | **26.98±6.59** | **Serum Vitamin D****(ng/ml)** |

Table (2) shows a significant low serum levels of vitamin D in patients than controls (fig.3).

**Figure (3): Comparison between asthmatic and control groups as regards serum Vitamin D.**

**Table (3): Comparison between Cases and controls as regards blood Eosinophilic count**:

|  |  |  |  |
| --- | --- | --- | --- |
| **Sig.** | **Control group** **(60)** | **Patient group** **(60)** |  |
| **P-value** | **Z** | **Mean±SD** | **Mean±SD** |  |
| **0.000****HS** | **-5.023** | **131.80±143.13** | **357.97±284.05** | **Eosinophilic count****(cells/mm³)** |

Table (3) shows a significant high levels of blood eosinophil count of patients than controls (fig.4).

**Figure (4): Comparison between asthmatic and control groups as regards blood Eosinophilic count.**

**Table (4): Comparison between Cases and controls as regards serum alkaline phosphatase levels:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sig.** | **Control group**  | **Patient group**  |  |
| **P-value** | **X2**  | **N (%)** | **N (%)** |  |
| **0.000****HS** | **28.35** | **22 (36.7%)****38 (63.3%)** | **54 (90%)****6 (10%)** | **ALP (IU)****High** **Normal** |

Table (4) shows that Mean serum level of alkaline phosphatase was significantly higher in patients than controls (fig.5).

 **Figure (5): Comparison between asthmatic and control groups as regards serum ALP.**

**Table (5): Comparison between Cases and controls as regards serum calcium levels:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sig.** | **Control group** **(60)** | **Patient group** **(60)** |  |
| **P-value** | **t** | **Mean±SD** | **Mean±SD** | **Calcium****(mg/dl)** |
| **0.299****NS** | **1.05** | **9.7±0.42** | **9.6±0.44** |

Table (5) shows that Serum calcium levels were normal in patients and controls with no significant difference (fig.6).

**Figure (6): Comparison between asthmatic and control groups as regards serum calcium.**

There were positive significant correlations between serum vitamin D and forced expiratory volume at 1 second (FEV1) (fig.7), forced vital capacity (FVC) andFEV1/FVC (fig.8)were detected.

25.00

50.00

75.00

100.00

**FEVI**

20.000

30.000

40.000

50.000

**VIT. D**

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**Figure (7): Correlation between vitamin D and FEV1.**

Figure (7) shows a positive significant correlation between serum vitamin D and FEV1.

50.00

75.00

100.00

125.00

**FEV1/FVC**

20.000

30.000

40.000

50.000

**VIT.D**

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**Figure (8): Correlation between vitamin D and FEV1/FVC.**

Figure (8) shows a positive significant correlation between serum vitamin D and FEV1/FVC.

**DISCUSSION**

In the current study 39 (65%) of patients were males and 21 (35 %) females with male to female ratio of 1.89:1, these data revealed that bronchial asthma was more common in males than females. This came in agreement with ***El Hefny et al.,*** ***(15)*** who showed a high prevalence of asthma in male children. Male to female ratio was 1.2:1 and the cause of this difference attributed to the finding that during early childhood boys have smaller airways at a given lung size than girls or maybe due to different environmental exposure patterns. ***Hossny et al., (16)*** reported that risk of developing asthma was higher for boys than girls with ratio 2.1:1. This could be attributed to sex-linked influence as male gender was a significant risk factor for asthma. Males have more allergen sensitization and show higher level of total immunoglobulin (IgE) than females.

Results of the current study show significant low levels of serum vitamin D in patients than controls. Studied patients showed that 8 (13.3%) had vitamin D insufficiency (Vit.D<30 ng/ml), 32(53.3%) had deficiency (Vit.D<20 ng/ml) and 20 (33.3%) had normal values (Vit.D=30-60 ng/ml), while controls 6 of them had (10%) deficiency and 54 (90%) had normal values. The mean value of vitamin D in asthmatic children was lower than controls.

These data came in concordance with multiple studies done among Egyptian children about status of vitamin D. **Baroncelli et al. (2008) *(17)*** reported that 37% of Egyptian children had vitamin D deficiency. **Prentice et al. (2009) *(18)*** showed that the average level of serum 25(OH) vitamin D was 25.3+10.3 which is deficient in Egyptian children. **Ghada El Hajj (2009**) ***(19)*** studied vitamin D status in North and South Africa. She stated that inappropriate level of vitamin D was due to limited sun exposure due to cultural practices, prolonged breast feeding without vitamin D supplementation, limited outdoor activity, lack of government regulation for vitamin D fortification of food, decreased maternal intake during pregnancy and increase the burden of infectious disease whereby utilization and turnover of vitamin D is increased.

In the current study there was decreased levels of serum vitamin D with increasing severity of asthma so that increase asthma severity was associated with much lower serum level of vitamin D. This came in agreement with ***Brehm et al., (30)*, *Manbir and Thomas,* *(31)*** who reported that asthmatic children with low serum level of vitamin D may have a greater risk of suffering severe asthma attacks than those with higher levels of the vitamin. They explained that vitamin D is a principal controller of innate immunity with the production of antimicrobial peptides able to kill viruses, bacteria and fungi. It exerts inhibitory effect on the inflammatory response to viral infections. Similar results were obtained by ***Laurie, (32)*,** who found that low vitamin D levels were significantly associated with elevated total IgE which is one of the markers of atopic sensitization and elevated eosinophil count which is increased in allergy.

Results of the current study show that there was significant higher family history of bronchial asthma, higher history of allergic rhinitis in asthmatics compared to control group. Similar to our results, ***Yagawa et al., (20)***found that airway responsiveness was increased in infants with a family history of asthma. Also ***van der Werff et al., (21)*** found that a family history of atopic diseases and allergic sensitization were predictors for asthma development. This can be explained by the fact that atopic sensitization is a risk factor for the development of upper and lower respiratory symptoms ***(22)***. Genetics also play an important causative role, as indicated by familial aggregation and the identification of candidate genes and chromosomal regions linked to asthma risk. Using a positive family history of asthma to identify children at increased risk could provide a basis for targeted prevention efforts, aimed at reducing exposure to environmental risk factors. ***(23)***

Results of the current study show a lower sun exposure in patients compared to control group. The primary source of vitamin D is from the skin’s production upon exposure to sunlight; secondary sources are eating vitamin D-rich and vitamin D enriched foods. ***(24)*** Similar to our study ***Uysalol et al., (25)*** study showed that asthmatic children had less exposure to sunlight compared to control group (p < 0.001). This is explained by the fact that families wanted to keep their children at home for fear of an asthma attack if they went outdoors or engaged in physical activities. ***Bener et al., (26)*** found similar results (p = 0.006). On the other hand, ***Bose et al., (27)*** found no significant difference between asthmatic children and control sun exposure with approximate three hours of exposure to sunlight per day (p = 0.49); however, the children in that study were dark-skinned and lived in an urban environment.

The present study showed that 20 (33.3%) patients were under weight, 28 (46.7%) normal weight and 12 (20%) obese while controls were 32(53.3%) under weight, 26 (43.3%) normal weight and 2 (3.3%) obese respectively. Statistical analysis revealed a significantly higher BMI centiles in patients than controls. Similar to our results, ***Ford, (28)*** in a meta-analysis has reported a relationship between asthma and obesity among adults, however, he found that studies conducted among children, have produced conﬂicting results. ***Gennuso et al., (29)*** found that in children, a cross sectional study have shown that excess body weight is associated with a higher rate of both symptoms and diagnosed asthma.

***Ehlayel et al., (30)*** showed that there is a significant difference between asthmatic children and healthy group in their study. They found that their patients were overweight and obese more than controls. They suggested that their asthmatic patients were obese since they avoid exercise that may trigger their symptoms. Also, ***Wang et al., (31)*** found a significant association between obesity and diagnosed asthma [(aOR) = 1.28; 95% confidence interval (CI): 1.02-1.60].

On the other hand, ***Beuther et al., (32)*** found no association between obesity and asthma with no significant difference between asthmatic children and control regarding their weight and BMI. Also ***Lavoie et al., (33)*** found in their study, a total of 139 (36%) patients had a normal BMI; 149 (39%) patients were overweight; and 94 (25%) patients were obese. There was no relationship between BMI and asthma when controlling for age and sex. ***Takami et al., (34)*** found no significant differences in weight or body mass index were observed between asthmatic children and control healthy subjects.

Results of the current study show a significant difference between patients subgroups regarding pulmonary function tests with significantly decreasing EFV1, FVC and FEV1/FVC % with increasing severity. Similar to our results ***Bacharier et al., (35)*** found that FEV1/FVC decreased as asthma severity increased (p < 0.0001); however unlike our results they found that FEV1 was generally normal, even in severe persistent childhood asthma.

**CONCLUSION**

The results of the present study showed that asthmatic children had lower levels of vitamin D than healthy controls. Lower levels of vitamin D were associated with high asthma severity and reduced asthma control. lower levels of vitamin D were reported in combined allergies.

**RECOMMENDATIONS**

Vitamin D supplementation for prevention of its deficiency. Adequate exposure to sunlight as a natural supplement of vitamin D should be encouraged for all children. Further studies are needed to asses asthmatic patients pre and post vitamin D supplementation to assess the reversibility of vitamin D deficiency effects on asthmatic patients. Further studies are needed to determine the relationship between measured vitamin D level and both asthma severity and allergy in childhood.

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**دراسة العلاقة بين نسبة 25-هيدروكسي فيتامين ( د ) في الدم وشدة حساسية الصدر عند الأطفال**

**المقدمة:**

 حساسية الصدر من الإمراض الشائعة في الأطفال والتي تصيب الجهاز التنفسي بضيق في المجاري الهوائية والتي تصبح ملتهبة وممتلئة بالكثير من الإفرازات. وغالبا ما تكون حساسية الصدر استجابة لواحدة أو أكثر من المثيرات. هذا الضيق إلى ظهور أعراض مثل أزيز في الصدر وصعوبة في التنفس وكحة مستمرة.

 على الرغم من انه لم يثبت حتى الآن وجود دور مؤكد لفيتامين( د) في حدوث حساسية الصدر إلا انه يرتبط بحساسية الصدر من عدة طرق منها انه يؤثر على الجهاز المناعي للإنسان وانه أيضا يؤثر على وظائف الرئتين.

**الهدف من الدارسة:**

قياس مستوى نسبة 25هيدروكسي فبتامين (د) في الدم البشري كإحدى دلائل شدة حساسية الصدر عند الأطفال.

**المنهجية:**

**تم تقسيم أفراد العينة إلى مجموعتين رئيسيتين:**

**المجموعة المصابة بحساسية الصدر:**

تشمل هذه المجموعة ستون طفلاً مصاباً بحساسية الصدر والذين يترددون على عيادة حساسية الصدر بمستشفى الأطفال بكلية الطب-جامعة عين شمس والذين تتراوح أعمارهم من 6 إلى 12 عاماً وتم تقسيم هذه المجموعة إلى ثلاثة مجموعات فرعية بناءاً على شدة المرض بسيطة مستمرة, متوسطة مستمرة **و**شديدة مستمرة وكل مجموعة تتألف من عشرين طفلاً.

**المجموعة الضابطة :**

 وتشمل ستون طفلاً من الأطفال الأصحاء الذين لا يعانون من حساسية الصدر.

كل الحالات خضعت لأخذ تاريخ شامل للمرض, فحص طبي سريري مفصل,اختبارات وظائف الرئتين وأبحاث معملية مثل صورة دم كاملة ( ونسبة الخلايا الإيزينوفيلية) ونسبة 25-هيدروكسي فيتامين (د) في الدم.

**نتائج البحث:**

* بلغت قيمة متوسط فيتامين (د) أقل بكثير لدى الأطفال المصابين بالربو من الضوابط.
* كان متوسط قيمة الفوسفاتيز القلوي أعلى بكثير لدى الأطفال المصابين بالربو من الضوابط.
* توجد علاقة بين نقص فيتامين(د) وشدة الربو في ما بين المرضى حيث أنه كلما نقص فيتامين (د) زادت شدة الربو.
* توجد علاقة بين انخفاض مستويات فيتامين (د) في الدم واستخدام مستنشق الكورتيكوستيرويد في المرضى.
* توجد علاقة بين التعرض لأشعة الشمس ونقص فيتامين (د) حيث أنه كلما قل التعرض لأشعة الشمس انخفض مستوى فيتامين (د) في الدم.

**الخلاصة:**

تبين من الدراسة وجود علاقة قوية بين مستوى فيتامين (د) في الدم وشدة حساسية الصدر عند الأطفال.

**توصيات البحث:**

* إعطاء الأطفال والمراهقين جرعات من فيتامين (د) يجب أن يوضع في الأولويات للوقاية من نقصه.
* تشجيع الأطفال على التعرض المعتدل لأشعة الشمس كمصدر طبيعي لفيتامين (د).
* التوصية بعمل أبحاث جديدة عن العلاقة بين فيتامين (د) وشدة حساسية الصدر والحساسية بوجه عام عند الأطفال.
* التوصية بعمل مزيد من الأبحاث عن جدوى علاج حساسية الصدر عند الأطفال بجرعات محددة من فيتامين (د).

**الكلمات الافتتاحية:**

حساسية الصدر عند الأطفال- 25-هيدروكسي فيتامين د- فيتامين د- وظائف الرئة.