

Original article

Serum vitamin D and IgE levels in infants and children under 2 years of age with recurrent chest wheeze

Background: Wheezing is a very common complaint on admission to the pediatric emergency department. There is an increasing awareness of the important role of vitamin D (VD) in the maintenance of the immune system, recurrent wheezing and respiratory health. **Objective:** The study aimed to estimate serum 25 hydroxy vitamin D (25OHD), IgE levels and blood eosinophilic count in infants and children under 2 years of age with recurrent wheeze. **Methods:** This case-control study was carried out on 85 infants (58 males and 27 females; as the patients' group, ranging in age from 6-24 months, diagnosed to have recurrent wheeze (>3 attacks), recruited from the Pediatric Emergency Department in comparison to 85 age and gender matched healthy infants with no history of wheeze (as the control group). Blood samples were taken from both groups to determine serum 25OHD level, IgE level, and eosinophil count. **Results:** Serum 25OHD levels of patients were significantly lower than those of controls ($p = 0.001$), whereas serum IgE and eosinophil counts of patients were significantly higher than those of controls ($p < 0.0001$ for both). Serum levels of 25OHD correlated negatively with the number of wheeze attacks and hospitalization. **Conclusion:** The study findings revealed lower serum 25OHD levels in infants with recurrent wheeze and provides additional evidence supporting the hypothesis that VD has a role in infant wheeze. VD supplementation might be practical and favorable for better control of recurrent infant wheeze.

Keywords: Vitamin D, IgE, Infants, Wheeze.

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INTRODUCTION

Wheezing is a very common complaint on admission to the pediatric emergency department and it is one of the most common causes of morbidity and mortality worldwide¹. Thirty percent of children have at least one wheezing attack before the age of three, and 50% before the age of six. It has been reported that recurrent wheezing attacks might frequently be seen before school-age and 40% of children with recurrent wheezing attacks can suffer from wheezing symptoms later in life². The relationship between wheezing in infancy and ensuing development of asthma has been under investigation for a long time. There is also detailed research determining the risk factors of recurrent wheezing³.

Vitamin D (VD) is a vital nutrient obtained either through endogenous production in the skin with exposure to ultraviolet B radiation or from dietary sources. It acts as a hormone and is well-known for its role in calcium and phosphorus homeostasis and skeletal health⁴. There is an increasing awareness of the important role of VD in

the maintenance of the immune system and respiratory health^{5,6}. The diseases traditionally associated with VD deficiency are rickets and osteomalacia, but growing data suggests that VD plays an important role in the lung development and vitamin D deficiency may be a risk factor for asthma, recurrent wheezing and respiratory infections⁷⁻¹⁰. The exact association between VD levels and recurrent wheezing is not obvious¹¹. There are a few studies investigating the relationship between recurrent wheezing and VD levels and these studies reported the association between maternal VD intake and early infant wheezing^{12,13}.

Although the impact of VD on the immune system is controversial, some researchers have claimed that the beneficial effects of stimulating VD pathway include decreased inflammation and enhanced defense against pathogens^{7,9,14-16}. VD is recognized as an important modulator of both the innate and adaptive immune system¹⁷. Increased susceptibility to infections, asthma, atopy, recurrent wheeze and reduced lung volume was noticed in patients with VD deficiency¹⁸⁻²¹.

VD deficiency has been largely attributed to dietary, lifestyle and behavioral changes during recent decades^{22, 23} especially in Western Countries. Both children, pregnant and lactating women are identified as groups vulnerable to VD deficiency²⁴. Moreover, both low maternal VD intake during pregnancy and low umbilical cord blood VD levels have been associated with increased risk of childhood wheezing^{12, 25}.

The possibility that immune mechanisms may contribute to the beginning of wheeze has been supported by studies that tend to favor an IgE mediated hypersensitivity type²⁶. Some authors have found higher total IgE levels in young children with persistent wheezing²⁷. A prospective population study has also shown that children with early sensitization and persistent wheezing had high IgE levels throughout childhood²⁸. Growing evidence suggests role for VD in the regulation of IgE and the development of allergic sensitization²⁹. Hence this study was conducted to: measure serum 25 hydroxy vitamin D (25OHD) and total IgE levels in infants and children under 2 years of age who presented with recurrent wheeze in comparison to healthy controls. To assess the risk factors for recurrent wheeze during this age period and investigate whether subnormal levels of 25OHD were associated with increased risk for recurrent wheeze. To investigate the relation between the studied parameters and the number of wheeze attacks and hospitalization in patient group.

METHODS

Study design and population

This single-center case control study was carried out in Assiut University Pediatric Hospital. The investigational protocol was approved by the local Ethical Committee of Medical School of Assiut University. Parents of all patients and controls gave their informed consents and written approvals for their children's participation before their enrollment in the study.

The study group consisted of 85 infants (58 males and 27 females), with a physician-diagnosed recurrent wheezing (>3 attacks) and had been admitted to the Pediatric Emergency Department. Inclusion criteria were; aged 6-24 months, normal growth and development consistent with age, no use of systemic or inhaled steroid during or just prior to the study, no history of other disease that could be related with wheezing. The diagnosis, severity and control level of wheezing were assessed in compliance with GINA criteria³⁰.

Exclusion criteria were; prematurity (<37weeks), low birth weight (<2500 gram), history

of NICU admission with RD, history of intubation and assisted ventilation during the neonatal period; patients who had an underlying cardiopulmonary, immunodeficiency, neurologic or metabolic diseases, or any chronic disorder. Further exclusion criteria were current infection, malnutrition and VD supplementation in the last 3 months.

The control group included 85 age- and gender-matched healthy infants randomly recruited from the Well Child Outpatient Clinic during their regular follow-up. They had no instance of chest wheeze, chronic disease or chest deformity; no diagnosed atopic dermatitis, allergic rhinitis or urticaria; no food or drug allergy; no diagnosed liver disease; no instances of lower respiratory tract infection in the last 3 months; no history of infection in the last 2 weeks; and no long-term supportive VD treatment in the last 3 months.

Each parent was informed about wheezing and was asked whether their infant had had this before, and if yes, how often. The wheezy infant was accepted as having >3 wheezing attacks with no known reason within the previous 6 months. The discrimination of wheezing was carried out in accordance with the advice of the European Respiratory Society Task Force.³¹

Parents of all cases and controls were asked to complete the same standardized questionnaire after face-to-face interviews. The questionnaire examined the patients' address and birth details; the family's socioeconomic conditions; the presence of asthma, allergic rhinitis, and eczema; presence of risk factors for wheezing, how many wheezing attacks they had, admission to emergency department in the last 6 months, consanguinity, contact with house birds and pets, passive smoking exposure, birth information, type of feeding during first 6 months and VD supplementation.

Full physical examination was carried out for all patients and controls with recording of the vital signs, patients' height, weight and BMI.

Sample collection and laboratory investigations:

Sample collection: 5ml of venous blood were collected from all patients and controls under standardized conditions after a fasting period of at least 4 hours. Two ml of blood were drawn on K3EDTA for complete blood count using the Coulter counter technique. (Coulter Hmx USA). The other portion of blood (3ml) was drawn in plain tubes, centrifuged (3,000 g for 10 min.) and the separated serum samples were divided and stored in aliquots at -20 °C until the day of analysis.

Assays for serum biomarkers:

Assay of serum 25OHD levels were done using EIA Kit from Immune diagnostic AG Bensheim, Germany, Ref.K2110. The results are expressed in nmol/L. According to the manufacturer's instructions of the Kit used, VD concentrations >75nmol/l are sufficient, from 30-75 nmol/l are insufficient, while <30nmol/l are seriously deficient. Assay of serum IgE levels was done using EIA from Bio-Check, USA, catalog no BC 1035. The results are expressed in IU/ml.

Statistical Methods

Clinical and laboratory data were statistically analyzed using a computer program (SPSS 16 for Windows). We calculated proportions and

percentages for qualitative variables and means and standard deviations for quantitative variables. Chi test, independent sample t-test, ANOVA and Pearson correlation and their non-parametric equivalents were used to test significant associations. We determined univariate associations between risk factors and the outcome of recurrent wheeze attacks using the Pearson χ^2 test for categorical variables and simple ordinal logistic regression for ordinal variables. Log transformation was performed to normalize data with skewed distribution. All of the P values are 2-tailed, with P<0.05 considered as statistically significant.

RESULTS

Table 1. Socio-demographic characteristics of patients and controls.

| Variables | Cases | Controls | Pearson Chi-Square | P-value |
|-----------------------|-----------------|-----------------|--------------------|---------|
| Age# (Mean \pm SD): | 0.98 \pm 0.16 | 0.97 \pm 0.08 | 0.554* | 0.581 |
| Sex: No (%) | | | 0.927 | 0.422 |
| -Males | 58 (68.2) | 52 (61.2) | | |
| -Females | 27 (31.8) | 33 (38.8) | | |
| Residence: No (%) | | | 0.443 | 0.614 |
| -Urban | 24 (28.2) | 28 (32.9) | | |
| -Rural/Slum | 61 (71.8) | 57(67.1) | | |

#Log transformation for age in months was performed to normalize the distribution then independent sample t-test was used.

* t- value of the independent sample t-test.

Table 2. Comparison of serum 25 OHD and total IgE levels, and eosinophil and WBC counts of the patient and control groups.

| Variables | Patients | Controls | P-value [§] | 95% CI |
|--|------------------|------------------|----------------------|----------------|
| -WBC (1000/mm ³) | 11.1 \pm 3.8 | 11.4 \pm 4.4 | 0.92 | -1.515-1.00419 |
| -Eosinophil count (cells/mm ³) | 343 \pm 366 | 143 \pm 160 | 0.0001* | 113.65-284.96 |
| -Total IgE(IU/mL) | 154.1 \pm 37.3 | 23.4 \pm 4.9 | 0.0001* | 58.301-203.219 |
| -25OHD(nmol/L) | 59.8 \pm 23.9 | 108.3 \pm 45.8 | 0.0001* | -59.473-37.445 |

§ Mann-Whitney Test was used as data was not normally distributed.

* Significant -Values are expressed as Mean \pm SD.

Table 3. VD statuses of patients and controls.

| VD levels (nmol/L) | Cases No. / (%) | Controls No. / (%) | P-value* |
|----------------------|-----------------|--------------------|----------|
| -Seriously deficient | 12 (14.1) | 00 (00.0) | 0.00001 |
| -Insufficient | 48 (56.5) | 28 (32.9) | |
| -Sufficient | 25 (29.4) | 57 (67.1) | |
| -Total | 85 (100) | 85 (100) | |

* χ^2 test was used.

Table 4. The relation between the investigated parameters and the number of hospitalizations of the patient group.

| Reference group | Hospitalizations | WBC (P-value) | Eosinophil count (P-value) | IgE (P-value) | VD (P-value) |
|-----------------|------------------|---------------|----------------------------|---------------|--------------|
| >3 times | None | .030* | .337 | .008* | .112 |
| | Once | .001* | .236 | .000* | .608 |
| | 2-3 | .002* | .232 | .381 | .084 |
| 2-3 times | None | .151 | .013* | .037* | .000* |
| | Once | .757 | .003* | .000* | .169 |
| Once | None | .087 | .026* | .071 | .014* |

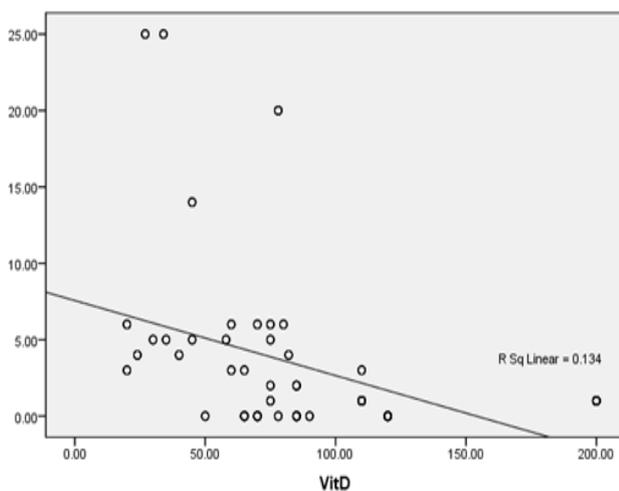
§ Log transformation for cell counts and biomarkers was performed to normalize the data distribution then one way ANOVA post hoc test was used.

*Significant.

Table 5. The correlation of the investigated parameters to the number of wheeze attacks in patient group.

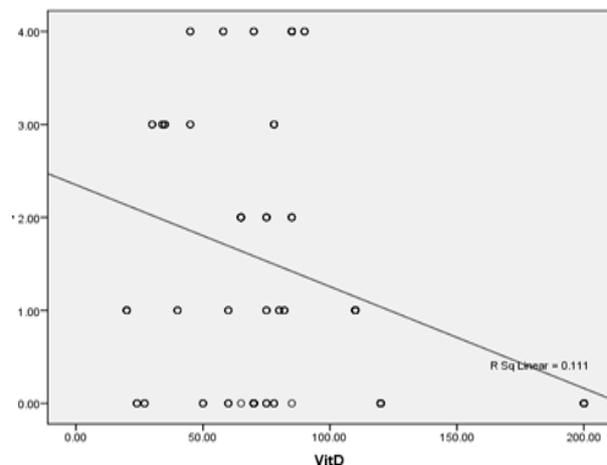
| Variables | Correlation coefficient# | P-value |
|--------------------------------------|--------------------------|---------|
| -Vitamin D (nmol/L) | - 0.4 | .0001* |
| -IgE(IU/mL) | 0.3 | .0001* |
| -Eosinophils(cells/mm ³) | 0.2 | 0 .035* |
| -WBC (1000/mm ³) | 0.08 | 0.2 |

#Spearman Correlation. * Significant



*spearman correlation was performed.

Figure 1. Correlation between serum 25OH D levels and number of wheeze attacks in the study group.



*spearman correlation was performed.

Figure 2. Correlation between serum 25OH D levels and number of hospitalization in the study group.

DISCUSSION

There are few studies investigating the relationship between recurrent wheezy chest and VD levels. The current study aimed to estimate serum VD and IgE levels in infants and children less than 2 years of age with recurrent wheeze and to investigate whether subnormal levels of VD are associated with increased risk for recurrent wheeze.

We found that serum VD levels were significantly lower in infants with recurrent wheeze than in healthy controls ($P=0.0001$). These findings are in agreement with those of Soner et al.⁴ who recommended that infants with recurrent wheezing should be scanned for VD insufficiency and they speculated that the duration of VD supplementation could be extended, especially for infants who had been diagnosed with recurrent wheezing during the first year of their life.

Also in consistent with the results of our study, Katarina et al.¹⁷ evaluated the risk factors for acute wheeze in preschool children and investigated whether subnormal levels of VD were associated with increased risk for acute wheeze. They found that preschool children with acute wheeze had significantly lower VD levels than healthy controls. Many other studies also concur that VD deficiency is associated with non-infectious wheezing illnesses in children^{3, 32, 34}. Other studies failed to prove this relationship^{11, 33}. Özyayın, et al¹¹ could not find any relationship between serum VD levels and recurrent wheeze probably due to the small sample size of their study.

A similar observation was reported from the United States, where no association was found between VD levels and exacerbations in asthmatic children, although asthma was more severe in those with VD insufficiency³². Moreover, Hibbs, et al.¹⁴ mentioned that there are some potential mechanisms by which VD supplementation may increase wheezing, and postulated that exposing the immature immune system to VD may predispose to asthma and allergy later in life.

Among the studied patients, 14.1% had serum 25OHD levels <30 nmol/L (seriously deficient), and 56.5% had levels from 30-70 nmol/L (insufficient) whereas in healthy controls, none had serum VD levels <30 nmol/L and 32.9% were VD insufficient. Hence, the patient group had a significantly higher proportion of infants with seriously deficient and insufficient serum VD levels than controls ($P=0.00001$ for both). Abdulbari et al.³⁵ found 23.4% of wheezy children and 10.5% of healthy children severely VD deficient. Moreover, they found nearly half (48.6%) of their healthy children mildly VD deficient. They concluded that

VD deficiency is highly prevalent even in sun-replete areas of the world and that VD supplementation and fortification of foods were inadequate to prevent its deficiency.

The possibility that immune mechanisms may contribute to the beginning of wheeze has been supported by studies that tend to favor an IgE mediated hypersensitivity type of mechanism.²⁶ Growing evidence suggests a role for VD in the regulation of IgE and the development of allergic sensitization.²⁹ In this study, serum IgE levels and eosinophil counts of patient group were significantly higher than those of healthy controls ($p<0.0001$ for both). Moreover, VD correlated significantly and negatively with serum IgE levels ($R= -0.331$, $P=0.0001$). Brehm et al.³⁴ reported a similar relationship and found that each 10-ng/mL increase in VD resulted in a 25-IU/mL decrease in IgE levels for asthmatic infants. Soner et al.⁴, however, found a positive linear relationship in their patients' group but a negative, albeit insignificant one, (log10) in their control group. They attributed this to the insufficient number of their controls and postulated that VD and IgE have a strict interaction with each other.

In this study, the relationships between the studied parameters (serum 25OHD, IgE and WBC & eosinophil counts) and the number of wheeze attacks and hospitalizations were investigated in the patient group. Serum VD levels showed highly significant negative correlation with the number of wheeze attacks indicating that VD deficiency having a significant role in the recurrence of wheezy chest in those patients.

This is consistent with Metin et al.³ who found significant relationship between recurrence and temporal pattern of wheeze with serum VD. It has also been reported that there is an association between VD deficiency and the number of asthma attacks in children with asthma³⁶. In agreement with our results, some studies have shown a relationship between lower VD levels and hospitalization³. A large cohort study for children with asthma in Costa Rica showed an association between low VD levels and asthma severity in terms of hospitalizations, medication use and airway responsiveness³⁴. However, a study from Canada failed to show this relationship³⁷.

Our study showed lower serum VD levels in infants with recurrent wheeze than in healthy controls. VD insufficiency was common in the study group whereas severe VD deficiency was significantly higher in infants with recurrent wheeze than in healthy controls. Therefore, this study provides additional evidence supporting a

role of VD in wheezing infants. It may be useful that all infants with recurrent wheezing be scanned for VD insufficiency. The role of VD supplementation in the control of recurrent infant wheeze awaits randomized controlled clinical trials.

REFERENCES

1. **SAGLANI S, BUSH A.** The early-life origins of asthma. *Curr Opin Allergy Clin Immunol* 2007; 7:83-90.
2. **MARTINEZ FD.** What have we learned from the Tucson Children's Respiratory Study? *Paediatr Respir Rev* 2002; 3:193-97.
3. **METIN U, EZGI PU, YASIN Y, GUNES P, TULIN AO, HAYRIYE VE, ET AL.** Serum level of vitamin D and trace elements in children with recurrent wheeze: A cross-sectional study. *BMC Pediatrics* 2014; 14:270.
4. **SONER D, SUKRU NG, MEHMET HC, RECEP S.** Is vitamin D insufficiency to blame for recurrent wheezing? *Int Forum Allergy Rh* 2014; 4 (12): 980-85.
5. **BLACK PN, SCRAGG R.** Relationship between serum 25-hydroxyvitamin D and pulmonary function. *Chest* 2005; 128:3792-8.
6. **HOLLAMS EM, HART PH, HOLT BJ, SERRALHA M., PARSONS F, DE KLERK NH, ET AL.** Vitamin D and atopy and asthma phenotypes in children: A longitudinal cohort study. *Eur Respir J* 2011; 38:1320-7.
7. **LANGE NE, LITONJUA A, HAWRYLOWICZ CM, WEISS S.** Vitamin D, the immune system and asthma. *Expert Rev Clin Immunol* 2009; 5:693-702.
8. **LIU PT, STENGER S, TANG DH, MODLIN RL.** Cutting edge: Vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. *J Immunol* 2007; 179:2060-3.
9. **KARATEKIN G, KAYA A, SALIHOGLU O, BALCI H, NUHOGLU A.** Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. *Eur J Clin Nutr* 2009; 63:473-7.
10. **GINDE AA, MANSBACH JM, CAMARGO JR CA.** Vitamin D, respiratory infections and asthma. *Curr Allergy Asthma Rep* 2009; 9:81-7.
11. **EDA ÖZAYDIN, MEHMET FB, BAHAR ÇÇ, GÜLŞEN K.** The Association between Vitamin D Status and Recurrent Wheezing. *Indian J Pediatr* 2013; 80 (11):907-10.
12. **CAMARGO JR CA, RIFAS-SHIMAN SL, LITONJUA AA, RICH-EDWARDS JW, WEISS ST, GOLD DR, ET AL.** Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 year of age. *Am J Clin Nutr* 2007; 85:788-95.
13. **DEVEREUX G, LITONJUA AA, TURNER SW, CRAIG LC, MCNEILL G, MARTINDALE S, ET AL.** Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007; 85:853-9.
14. **HIBBS AM, BABINEAU DC, WANG X, REDLINE X.** Race differences in the association between multivitamin exposure and wheezing in preterm infants. *J Perinatol* 2015; 35:192-97.
15. **CHEN S, SIMS GP, CHEN XX, GU YY, LIPSKY PE.** Modulatory effects of 1, 25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* 2007; 179 (3): 1634-47.
16. **HANSDOTTIR S, MONICK MM, LOVAN N, POWERS L, GERKE A, HUNNINGHAKE GW.** Vitamin D decreases respiratory syncytial virus induction of NF-kappa B-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol* 2010; 184(2): 965-74.
17. **KATARINA SH, GUNILLA H, JON R.K, BJÖRN N, INGER K, CHRISTIAN GG, ET AL.** Sub-normal levels of vitamin D are associated with acute wheeze in young children. *Acta Pædiatr* 2014; 103: 856-61.
18. **JOLLIFFE DA, GRIFFITHS CJ, MARTINEAU AR.** Vitamin D in the prevention of acute respiratory infection: systematic review of clinical studies. *J Steroid Biochem Mol Biol* 2013; 136: 321-29.
19. **LITONJUA AA.** Vitamin D deficiency as a risk factor for childhood allergic disease and asthma. *Curr Opin Allergy Clin Immunol* 2012; 12: 179-85.
20. **PAUL G, BREHM JM, ALCORN JF, HOLGUIN F, AUJLA SJ, CELEDON JC.** Vitamin D and asthma. *Am J Respir Crit Care Med* 2012; 185:124-32.
21. **ZOSKY GR, BERRY LJ, ELLIOT JG, JAMES AL, GORMAN S, HART PH.** Vitamin D deficiency causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med* 2011; 183: 1336-43.
22. **NOUR B, PATRICIA DM, JOHN DW, JEAN-CLAUDE S, ISABELLA AM.** Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *J Allergy Clin Immunol* 2014; 133:147-53.
23. **HOLICK MF.** Vitamin D deficiency. *N Engl J Med* 2007; 357:266-81.
24. **MULLIGAN ML, FELTON SK, RIEK AE, BERNAL-MIZRACHI C.** Implications of vitamin D deficiency in pregnancy and lactation. *Am J Obstet Gynecol* 2010; 202: 429, e1-9.
25. **CAMARGO JR CA, INGHAM T, WICKENS K, THADHANI R, SILVERS KM, EPTON MJ, ET AL.** Cord blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics* 2011; 127:e180-e187.
26. **LOPEZ N, DE BARROS-MAZON S, MARLUCE DOS SANTOS VILELA, M, CONDINO NETO A, RIBEIRO JD.** Are immunoglobulin E levels associated with early wheezing? A prospective study in Brazilian infants. *Eur Respir J* 2002; 20: 640-45.

27. **CLOUGH JB, KEEPING KA, EDWARDS LC, FREEMAN WM, WARNER JA, WARNER JO.** Can we predict which wheezy infants will continue to wheeze? *Am J Respir Crit Care Med* 1999; 160: 1473-80.
28. **HILL R, WILLIAMS J, TATTERSFIELD A, BRITTON J.** Change in use of asthma as a diagnostic label for wheezing illness in school children. *BMJ* 1989; 299: 898.
29. **SANDHU MS, CASALE TB.** The role of vitamin D in asthma. *Ann Allergy Asthma Immunol* 2010; 105:191-202, 17.
30. **VAN WEEL C, BATEMAN ED, BOUSQUET J, REID J, GROUSE L, SCHERMER T, ET AL.** Asthma management pocket reference 2008. *Allergy* 2008; 63:997-1004.
31. **BRAND PL, BARALDI E, BISGAARD H, BONER AL, CASTRO-RODRIGUEZ JA, CUSTOVICET A.** Definition, assessment and treatment of wheezing disorders in preschool children: An evidence-based approach. *Eur Respir J* 2008, 32:1096-1110.
32. **BREHM JM, SCHUEMANN B, FUHLBRIGGE AL, HOLLIS BW, STRUNK RC, ZEIGER RS, ET AL.** Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol*.2010; 126: 52-8.
33. **RAZI CH, AKIN O, HARMANCI K, AKIN B, RENDA R.** Serum heavy metal and antioxidant element levels of children with recurrent wheeze. *Allergol Immunopathol*, 2011; 39:85-9.
34. **BREHM JM, CELEDON JC, SOTO-QUIROS ME, AVILA L, HUNNINGHAKE GM, FORNO E, ET AL.** Serum VD levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med* 2009; 179(9): 765-71.
35. **ABDULBARI B, MOHAMMAD SE, HALE ZB, QUTAYBA H.** The impact of VD deficiency on asthma, allergic rhinitis and wheezing in children: An emerging public health problem. *J Fam Community Med* 2014, (21) 3:154-61.
36. **CHINELLATO I, PIAZZA M, SANDRI M, PERONI D, PIAGENTINI G, BONER AL.** Vitamin D serum levels and markers of asthma control in Italian children. *J Pediatr* 2011; 158:437-41.
37. **ROTH DE, JONES AB, PROSSER C, ROBINSON JL, VOHRA S.** Vitamin D status is not associated with the risk of hospitalization for acute bronchiolitis in early childhood. *Eur J Clin Nutr* 2009, 63:297-9.