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Original Research Paper

Significance of Serum Vitamin D Level in Bronchial Asthma Patients

Medical Science

KEYWORDS : BRONCHIAL ASTHMA, VITAMIN D, HYPOVITAMINOSIS D

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ABSTRACT

***Objectives**: To determine serum vitamin D level in patient of Bronchial Asthma and compare it with controls.*
***Subjects**: Patients of age 21-60 years with diagnosis of Bronchial Asthma on the basis of clinical and radiological sign and symptoms with spirometric criteria (FEV1/FVC - <0.7 ,% FEV1 <80%) with significant bronchodilatation. Controls were healthy subjects of age 21-60 years. 102 patients of Bronchial Asthma and 103 Controls were included in the study.*
Interventions: We measured total serum 25-(OH)-D by Diasorin competitive radioimmunoassay (RIA) (AID Diagnostika, GmbH, Strasburg, Germany.
Design of study: Single-center cross-sectional study.
***Results**: The mean Serum Vitamin 25-(OH)D of Bonchial Asthma patients was 22.8 ± 8.2 ng/mL,Among controls it was 30.5 ± 8.6 ng/mL.(p <0.05). This analysis significantly shows that mean serum vit D levels was low in Asthma as compared with Controls and there is no significant difference of serum VitD in various group like age, sex and indoor/outdoor activity.*
***Conclusions**: Our study concludes that Hypovitaminosis D was highly prevalent among patient of bronchial Asthma as compared to general population in same geographical area.*

Introduction

Vitamin D is responsible for intestinal absorption of calcium and phosphate. Dietary sources include liver, fish, egg, fish liver oil and dietary supplements. As very few foods contain vitamin D, sunlight exposure is the primary determinant of Vit D status in humans. The bone health consequences of vitD deficiency are well established, however, other disorders have also been linked to vitamin D insufficiency, including asthma. New research has identified VitD also play vital for respiratory health. Due to its anti-inflammatory properties, 1,25(OH)D may be very important in people with asthma. We summarize evidences that Vit D receptors expressed in multiple lung cell and acts to protection from asthma by several mechanisms including promoting lung immunity, decreasing inflammation and slowing cell cycling hyperplasia, The objective of this study was to systematically review the evidence for an epidemiological association between low serum levels of vitamin D and presence of asthma.

Methods

Patients of age 21-60 years with diagnosis of Brnchial Asthma on the basis of clinical and radiological sign and symptoms with spirometric criteria ($FEV_{1/}FV_C$ - <0.7 ,% FEV_1 <80%) with significant bronchodialatation.

Controls are healthy subjects of age 21-60 years free from any disease and morbidity.

All the subjects were divided into male/female, smokers/non smokers.

EXCLUSION CRITERIA

All known cases of autoimmune disease, active cancer,Morbid obesity,Diabetic patient, Chronic Kidney disease and osteomalacia .

INVESTIGATION REQUIRED

X ray chest, Spirometry, Serum vit D in form of 25 Hydroxy D using chemiluminescence method.

Sampling and Study Design:

A simple randomized sampling selection of Bronchial asthma included a total of 102 Bronchial asthma patients and 103 Controls.This study was an open- labelled, randomized, cross-sectional study.

Specimen collection & Processing

Under aseptic techniques, we performed routine venous puncture. Using a serum separator tube (SST; Becton Dickinson, Franklin Lakes, NJ, USA), 3mls non fasting whole blood was drawn and left to clot for about 30 minutes, followed by centrifugation for 12-15 minutes needed for analysis of vitamin D 25-(OH)-D.

Serum Vitamin D Analysis: We measured total serum 25-(OH)-D by Diasorin competitive radioimmunoassay (RIA) (**AID Diagnostika, GmbH, Strasburg, Germany**).Prior to analysis, the serum samples were precipitated and extracted with acetonitrile. After centrifugation, an aliquot of the supernatants were incubated with specific antibodies against 25(OH)D for 90 minutes and 2 hours respectively at an ambient temperature. After centrifugation, the supernatants were decanted and counted.

Definition of Vitamin D Status:

Vitamin D status was defined as:-Serum 25(OH)D levels ≤ 20 ng/mL was used to define vit D deficiency, serum 25(OH)D 20 – 30 ng/mL as insufficiency and >30 ng/mL defined sufficient vit D levels[1] (Gomez AC et al 2003).

Statistical analysis All analyses were performed with SPSS software for Windows, version 20 (SPSS Inc., IBM, Version 20.0, USA). p-<0.05 considered as significant.

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RESULTS

AGE AND SEX DISTRIBUTION

In our study 203 subjects included with age ranged from 20-60 years..All subjects divided into control and cases, Out of which 103 were controls including 61 males and 42 females with mean age 35.5 ± 12.7 years,12 smoker and 91 non smokers , 100 were case including 44 males and 56 females with mean age 33.2 ± 13.2 years, 5 smoker and 95 non smokers,90 outdoor and 10 indoor .

Frequency distribution of Serum Vitamin 25 – (OH) D in study groups-

The mean Serum Vitamin 25-(OH)D of ASTHMA patients was 22.8 ± 8.2 and among controls it was 30.5 ± 8.6 ng/mL with p-value of <0.05, this analysis significantly shows that mean serum vit D levels was low in BRONCHIAL ASTHMA as compared with CONTROLS.

Table.1 : Frequency Distribution of Serum Vitamin D Levels among study groups

Study Groups	CASES (vit D in ng/dL ±S.D.)	CONTROLS (vit D in ng/mL ± S.D.)	*t value*	p-value
ASTHMA	22.8±8.2	30.5±8.6	6.5	<0.05

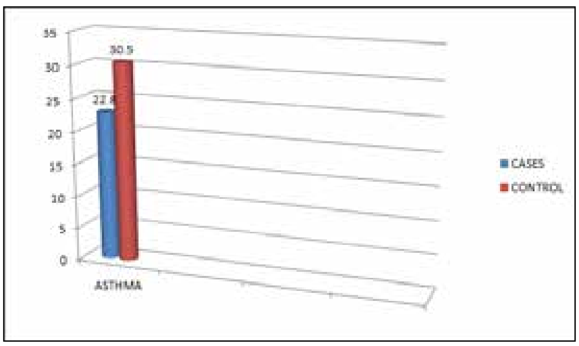

Figure.1- Frequency Distribution of Serum Vitamin D Levels among study groups.

Observation of Vitamin D Levels within a group with Respect to Sex, smoking status and outdoor and indoor status:

In this analysis among ASTHMA group male had serum vitD levels of 23.1±7.5 ng/mL and female had 22.6±8.8 with p-0.74*(not significant)*,smoker had 24.4 ± 7.3 ng/mL and non smokers have 22.7 ± 8.3 ng/mL with p-0.60*(not significant)*,outdoor had 23.1±8.3 and indoor had 21.1±7.7 with p-0.42*(not significant)*, and in control group male 31.3±8.4 and female had 29.6±9.0 ng/ml with p-0.33*(not significant)*., smoker have 27.2 ± 6.5 and non smoker have 31.0 ± 8.0 ng/mL with p value 0.15*(not significant)* respectively, which clearly show that relationship of serum vit D levels in any study groups is not significantly varies with confounders like sex, smoking and indoor/outdoor activity.

Study groups with Mean age in yrs	Epidemiological criteria	NO. of subjects	**VitD in ng/mL** ± S.D.	p value
Bronchial Asthma patients with mean age 33.25±13.2 yrs	Male	44	23.1±7.5	0.74
	Female	56	22.6±8.8	
	Smokers	5	24.4±7.3	0.60
	Non smoker	95	22.7±8.3	
	Outdoor	90	23.1±8.3	0.42
	Indoor	10	21.1±7.7	
CONTROL with mean age 35.0±12.7 yrs	Male	61	31.3±8.4	0.33
	Female	42	29.6±9.0	
	Smokers	12	27.2±6.5	0.154
	Non smoker	91	31.0±8.0	

Table.2- Observation of Vitamin D Levels within a group with Respect to Sex, smoking status and outdoor and indoor status

DISCUSSION

This study was an open-labeled, randomized, cross-sectional study to observe the serum levels of vitamin D among patients of Bronchial asthma attending at Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh. We analyzed serum vit D levels in first in Asthma and compared it with general population and we got good significant difference. We also compared serum vitD levels among these groups on the basis of Gender and smoking habits, and indoor and outdoor activety and we fail to find significant difference due to these confounding factors.

The Vitamin D Molecule

There are two forms of vitamin D, vitamins D_2 and D_3. Generally, vitamin D3 is endogenously produced in the skin in response to ultraviolet B (UvB) radiation. In 1997, the Dietary Reference Intakes for the Institute of Medicine[2] in the United States established the adequate intake (AI) of vitamin D for male and females at different ages, as depicted below in Table 2.

Table 3: The recommended adequate intake of vitamin D (IU/day) for males and females of various age groups

Age	Adequate intake (IU/day)	**Adequate intake(microgm/day)**
<50 years	200	5
51-70 years	400	10
>70 years	600	15

In Table 3,AI differ with respect to age. Studies of the 25(OH)D concentrations of older age groups have shown that recommended levels may be inadequate to maintain vitamin D sufficiency[3,4],

Normal Levels of Circulating Vitamin D

The most consistent criteria used to categorize vitamin D are <20 ng/mL, 20-30 ng/mL, and ≥30 ng/mL for deficiency, insufficiency, and sufficiently, respectively[5,6].

Vitamin D Metabolism

Since vitamin D is a fat-soluble molecule, it must be consumed with lipids for proper absorption. Dietary vitamin D is absorbed from a micelle with fat, and broken down with bile salts[7] Once vitamin D has been ingested and absorbed, or cutaneously produced and diffused through the skin, it enters circulation bound to DBP[8].As depicted in Figure 4, vitamin D undergoes two important hydroxylation reactions to become active hormonal form, first takes place in liver cells producing 25-hydroxyvitamin D [25(OH)D][7] and second reaction takesplace in kidneys where 25(OH) D undergoes hydroxylation into the biologically active metabolite1,25$(OH)_2$ D[9,10].

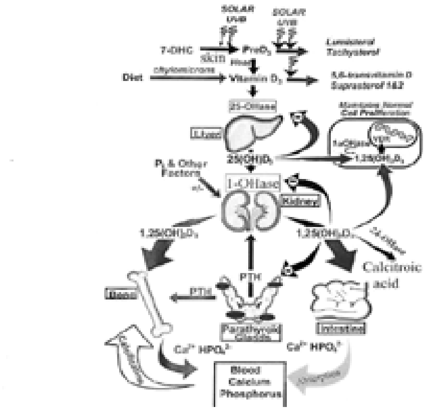

Fig-2. The cutaneous production and dietary absorption of Vit D, and its subsequent metabolism and regulation (25-0Hase, 25- hydroxylase; I-OHase, 1 a-hydroxylase).

Alternatively, some 25(OH)D molecules will re-enter circulation and bind to DBP[11,12].Although the kidneys are the primary site of l,25(OH)$_2$D production, extra-renal production takes place in more than thirty tissues, such as placental cells, bone cells, keratinocytes, macrophages, and intestinal cells[8,13,14].The 25(OH)D is the primary metabolite in circulation, while 1,25(OH)$_2$D, the biologically active form, is able to carry out its functions within target cells .

Mechanisms of Action

Vit D may act through both genomic and non-genomic pathways. The 1,25(OH)$_2$ D-DBP complex is taken up by the cell from circulation through diffusion or receptor-mediated endocytosis. Once inside, 1,25(OH)$_2$ D is released from DBP and forms a complex with the vitamin D receptor (VDR),mediating its effect on the target cell[15].These mechanisms involve the association of 1,25(OH)$_2$D with a membrane VDR that activates signal transductions such as calcium transport channels and mitogen-activated protein kinase (MAPK) activation[16,17].Association with cytoplasmic plasma membrane proteins such as tyrosine kinase receptor or G protein coupled receptors[15,17] may lead to phosphorylations and involvement of secondary messengers that carry out rapid responses in the liver, intestines, bone, parathyroid and pancreatic cells[7].

Genomic pathways take place as the 1,25(OH)$_2$D-VDR complex moves from the cytosol into the nucleus[11]. By binding to VDR, 1,25(OH)$_2$ D is able to regulate the transcription rates of more than sixty genes involved in mechanisms such as calcium balance, immune regulation and cell differentiation[18].These mechanisms potentially take place in more than 30 tissues in which nuclear VDRs have been isolated, including bones, intestines, kidneys, lungs, muscle and skin[7]. Biological actions resulting from these reactions include calcium homeostasis, mediation of 1,25(OH)$_2$D synthesis and breakdown rates, reduction in PTH production and regulation of immune responses[11].

Biological Functions

The association between vit D and bone metabolism has also been observed in studies of VDR gene polymorphisms and the development of osteoporosis[19]and rickets.However, involvement of vitD in the immune processes is less well understood.

Involvement of Vitamin D in Immune Processes

The immunomodulatory role of 1,25(OH)$_2$ D is primarily due to local production of this by immune cells such as macrophages, dendritic cells (Des) and lymphocytes, attenuating adaptive and enhancing innate immune mechanisms[20]. The production of VDR in these cells allows 1,25(OH)$_2$ D to regulate expression of cytokines and cell receptors.VitD primarily regulate innate immunity by interacting with macrophages, which express 1-hydroxylase[20]. Macrophages are cells that are responding to pathogen-associated molecular patterns (PAMPs) on the cell surface of microbes[21]. The PAMPs are recognized by pattern recognition receptors (PRRs) on the cell surface of macrophages[22]. The most important vitamin D associated innate immune response to infection is the release of the antimicrobial protein cathelicidin thereby increasing pathogen killing. Cathelicidin is an antimicrobial peptide, contributes to pathogen killing by coordination with transcription factors that promote autophagy[24]. Macrophages and DC$_s$, presenting antigens to T or B cells and activating them[25].Activated T and B lymphocytes both express VDR, and therefore allow for the effects of 1,25(OH)$_2$ D, such as suppressing the proliferation of B and T lymphocytes and reducing immunoglobulin production. The primary target is the T helper (Th) cell, which modulates cytokine production[20].T-cells differentiate into Th^1 and Th^2 lymphocytes. This polarization of the adaptive immune system influences production of various cytokines e.g.Interleukin (IL)-12, produced by macrophages and DCs.1,25(OH)$_2$ D inhibits IL-12 expression by macrophages and DCs[26].This is consistent with the commonly acknowledged role of 1,25(OH)$_2$D in immunosuppression, as the hormone has been shown to suppress the Th1 pathway and induce the Th^2 pathway, inhibiting inflammatory cytokines such as interferon (IFN)-y, tumour necrosis factor-a and IL-2, and increasing development of Th^2 cells and corresponding cytokines (IL-4, IL-5, IL-l0 and IL-13)[27].This interaction is beneficial in combating some chronic conditions, like autoimmune or inflammatory diseases.

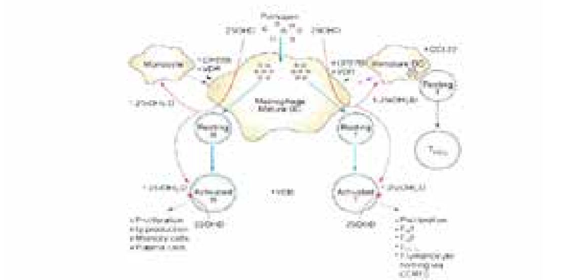

Fig-3. Interactions between vitamin D metabolites and the immune system

Role of vit D in lung immunity

The respiratory system is first line of defense and function as *physical* barrier to prevent the entry of inhaled pathogens and as an immune effectors. When the respiratory epithelium recognizes the presence of a pathogen it responds by releasing antimicrobials, chemokines and cytokines. Alveolar macrophages (AMs) recognize, phagocytose and remove inhaled material. They are activated either in response to pathogens or through an autocrine/paracrine response to cytokines. Activation leads to enhanced phagocytosis and killing of pathogens as well as coordination of both innate and adaptive immune responses. As discussed above VDR is constitutively expressed in Monocytes, activated macrophages, dendritic cells, natural killer cells, T and B cells. These activation has potent antiproliferative, prodiffrerentiative and immunomodulatory functions, both immune enhancing and immunosuppressive.

Dendritic cells use PRRs to monitor the local environment

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for pathogens. When a pathogen is encountered, it is ingested and its proteins are processed into peptides which are then presented at the surface of the dendritic cell. Activated dendritic cells produce chemokines and migrate to local lymph nodes where they present antigenic peptides bound to major MHC molecules to naïve T-cells (CD4and CD8+ T-cells) and induce their activation and differentiation. Dendritic cells thus serve as a link between innate and adaptive immune responses[28] .Vitamin D modulates dendritic cell maturation and chemokine profile and macrophage innate immune functions [20].Vit D has been shown to be metabolized by various other epithelial cell types and affect their functions. By influencing key innate immune effectors vitamin D may play an important role in how the lung recognizes and responds to pathogens.

ROLE OF VIT D IN BRONCHIAL ASTHMA

Asthma - A Complex Inflammatory Disease according to the American thoracic society's revised definition in 1987 **[ATS 1987]**, asthma is: "a clinical syndrome characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli[29].It is a complex heterogeneous disorder with respect to immunopathology, clinical phenotypes, response to therapies, and natural history [30] and characterized by chronic airway abnormalities such as airway hyper-responsiveness; airway remodeling, and infiltration of eosinophils and T-helper type^2(Th^2) cells in the airway sub-mucosa, which leads to inflammation and edema in the bronchial mucosa, and hyper-secretion of mucus[31].Today's growing asthma epidemic has prompted research to focus on various target immune cells involved in airway allergic inflammation, with needed emphasis on Th^2 cells and eosinophils[32]. Major cells involved are notably epithelial cells, nerve cells, smooth muscle cells, mast cells, DCs, eosinophils/basophils and Th^2 cells[33].

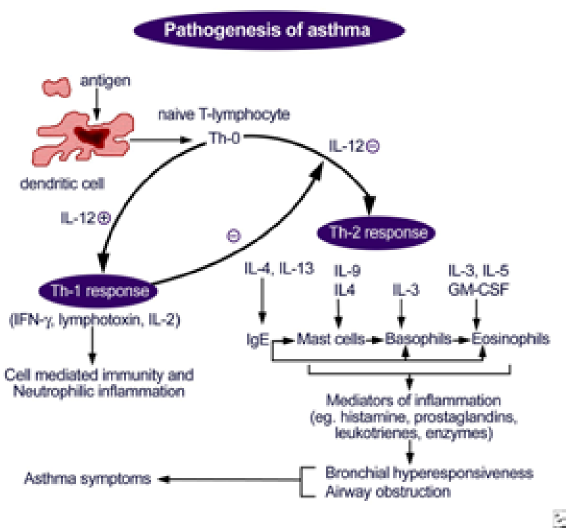

Fig.4-Roles of inflammatory cells and chemokines in asthma pathogenesis[34]

In clinical studies, increased eosinophil sputum counts associate with asthma exacerbations [35]. Furthermore, eosinophils are associated with lung tissue remodeling, a permanent consequence of chronic airway inflammation[36] Accordingly, eosinophils have been and remain a target in asthma pathogenesis and therapeutic research[37].

Epidemiology, Prevalence and risk factors of asthma

Asthma often develops in childhood, but it can develop at any stage in life. Factors that trigger asthma include indoor and outdoor allergens, air pollutants, respiratory tract infections, exercise, weather changes, food additives, drugs, and extreme emotions [38].The most common early life condition affecting children is wheezing[39]. Parental history of asthma, male sex, exposure to indoor air pollutants, respiratory syncytial virus (RSV) and human rhinovirus (HRV) infection are some of the risk factors associated with asthma in early childhood. Asthma is also associated with obesity, ethnicity, and living in westernized countries.CDC has estimate female sex, atopy, and low socioeconomic status are also risk factors for asthma in adults[40].According to hygiene hypothesis, decreased exposure to infections in early life leads to altered development of the immune system.As described, immune system of many people has deviated towards a predominantly Th^2 phenotype rather than a balanced Th^1/Th^2 response. This inadequate up-regulation of Th^1 immune responses is thought to result in increased risk of asthma and allergy. There are also dietary hypotheses explained that reduced dietary antioxidants (e.g.vitamin C,D and E), minerals (e.g. Se),and PUFA was associated with an increase in asthma. These hypotheses suggest that declining lung and respiratory epithelial antioxidant defenses increased oxidant induced airway damage, inflammation and damage[41].New research has identified 1,25(OH)$_2$D plays vital for respiratory health and in pulmonary tissues as it has been shown to be both anti-infectious and anti-inflammatory and thus may be important in the pathobiology of asthma whose exacerbations are commonly triggered by infection and chronicity of inflammation.

The Vitamin D Receptor (VDR) and Asthma Genetics

Cells that contain vit D receptors (VDR) are targets for Vit D activity[42].VDRs have been localized in both respiratory epithelial cells and in bronchial smooth muscle. Pulmonary VDRs have been proven to be fully functional: 25(OH)D is converted into 1,25(OH)D in respiratory epithelial cells and both VDR and CYP24A1 (a hydroxylase that metabolizes 1,25(OH)$_2$D) synthesis are increased in bronchial smooth muscle cells[43].Eight single nucleotide polymorphism of the VDR gene have also been associated with asthma and atopy in a large population-based study[44] thus establishing a relationship between asthma and Vit D metabolism genetics.

1,25(OH)D Abrogates Inflammation

As Inflammation is a key component in the pathology of asthma, 1,25(OH)$_2$D has been shown to be anti-inflammatory in many tissues including lung .When airway smooth muscle cells were treated with TNF alpha and/or IFN gamma to mimic the inflammation of an acute asthmatic flare, and then exposed to increasing doses of 1,25(OH)$_2$D and GC, inflammatory cytokine production decreased in. Both RANTES and IP-10 were noted to have significant decreases[45].

In yet study, airway epithelial cells were infected with RSV (respiratory syncitial virus), a common culprit of childhood lower respiratory tract infections marked by excessive inflammation of the respiratory epthelium, When the cells were pre-treated with Vit D, there was a dose-dependent increase of the NF-κB inhibitory protein IκBα[46].If 1,25(OH)$_2$D could decrease inflammation caused by viral infections in pulmonary tissues, it could also potentially prevent the onset of acute asthma exacerbations caused by a rapid increase of inflammation.

1,25(OH)$_2$D Decreases Hyperplasia and Cell Cycling

Chronic tissue changes in asthma results from hyperplasia of smooth muscle and glandular tissue. 1,25(OH)$_2$D ameliorates this disordered growth; in airway smooth muscle cells, VDR regulated genes that are also part of the asthmatic network include VGEF, a gene known for increasing smooth muscle hyperplasia, IL-6, a gene involved in increasing remodeling, and FN1 (fibronectin), which has been shown to be increase extracellular matrix deposition[47]. Down-regulating these genes would result in decreased remodeling. Decelerating the cell cycle can also result in more organized regeneration, as it allows cells more time to repair DNA damage. When airway smooth muscle cells that were sensitized with asthmatic serum were treated with $1,25(OH)_2D$, fewer cells proceeded into S phase[48].

All of these studies show that $1,25(OH)_2D$ reduces disorganized hyperplastic regeneration in pulmonary tissue. It has been suggested that Vit D may slow the progressive decline in lung function seen in some asthmatics, a characteristic of airway remodeling by inhibiting the formation of matrix metalloproteinase as well as fibroblast proliferation and influencing collagen synthesis. In airway remodeling, a certain type of smooth muscle growth occurs prominently which reflects airway inflammation and leads to the impaired lung function. In addition it may also modulate protease-antiprotease imbalance and oxidative stress [49].

Observational studies have suggested that Vit D plays a role in allergic airway diseases and that low serum Vit D is associated with poor asthma control, frequent and severe asthma exacerbations, reduced lung function, and increased medications use[50]. These findings led researchers to extensively study the role of vitamin D in asthma.

A study by Gupta *et al,* in 86 British children aged 6-16 years with varying asthma severity found that vitamin D levels were significantly lower in severe therapy resistant asthmatics, positively related to FEV1, FVC, and negatively associated with airway smooth muscle mass. This concluded that vit D supplementation may be helpful in management of steroid therapy resistant asthma in pediatric population[51].

A recent study by Sutherland *et al.,* in asthmatic adults observed an association between reduced Vit D levels and impaired lung function, increased airway hyper-responsiveness and reduced glucocorticoid response. It showed low Vit D levels affect long-term prognosis of asthma by increasing expression of pro-inflammatory TNF-α and accelerating deterioration of lung function[52].In a case-control study conducted on Iranian children between 6 and 18 years, serum 25(OH)D levels were linearly associated with FEV1 and FEV1/FVC, and low levels of VitD were associated with asthma[53].A randomized control study conducted on Japanese school children aged 6-15 years reported that a 1200 IU/day Vit D supplement taken during the winter and early spring helped prevent influenza and asthma attacks[54]. An ecological study using asthma prevalence data in adults from United States and Australia reported that the prevalence of asthma was associated with geographical latitude, implying that latitudinal difference in isolation and subsequently serum vitamin D as an environmental factor influences the prevalence of asthma.11 out of 15 study reported an inverse relationship between vitD levels and Asthma[55].

An Australian cohort study reported that an increased risk of asthma was observed in children born in winter and autumn compared to summer, suggesting that reduced sun exposure levels during pregnancy had resulted in low levels of Vit D in mothers, and resulted in asthma in their offspring[56] .

While most studies suggested low levels of VitD are associated with poorer respiratory health, some have also raised concerns about higher levels. A cohort study of Swedish children aged 6 years reported that higher Vit D intake was associated with more prevalent atopic manifestations, suggesting that VitD intake during infancy may cause atopic allergies later in childhood[57].Thus a connection between Vit D status and asthma has been considered since many years and Vit D deficiency has been blamed as one cause of increased asthma prevalence in the last decades[58]. Present study also clearly documents less serum vit D in asthmatic group as compared to normal population of same geographical area with significant P value <0.00001 even they have low mean age as compared to control group. out of total patients 56 are females and 44 are males in which 95 are non smokers and only 5 are smokers which documents two things, first asthma more prevalent in females and secondly most of patients are non smokers but still have very significant low vit D levels which further indicate that smoking have no much significant role on circulating vit D levels.Present study further supported by a population-based cross-sectional data by Black *et al.,* reported a positive association of serum vitamin D levels and lung function in adults[59].Likewise, a cross-sectional analysis of British birth cohort participants at the age of 45 years reported that increasing serum 25(OH)D levels were linearly associated with greater FEV1 and FVC[60].According to a cohort study conducted on Danish adults, lower plasma 25(OH)D levels were not only associated with lower FEV1 and FVC, but also with faster decline of lung function[61]. In a review, Zarogoulidis *et al.* reported that between 2007 to 2012, 11 out of 15 observational studies report an inverse association between serum 25(OH)D and asthma[62]. Searing et al also found that low levels of vitamin D were positively correlated with FEV1 percent predicted and the FEV1/forced vital capacity (FVC) ratio[63]. More recently, a high frequency of vitamin D insufficiency (35%) in 1024 North American children with mild-to-moderate asthma has also been reported. In addition, vitamin D insufficiency was associated with a lower mean forced expiratory volume in one second (FEV1) compared with children with sufficient levels. VitD supplementation decreased the number of asthma exacerbations triggered by acute respiratory tract infections[64].Even though the dose of vitamin D was inadequate to increase serum 25(OH)D levels, significant clinical benefits were observed. In contrast some authors have also suggested that vitamin D may promote, rather than ameliorate the asthmatic phenotype[65].Regular vitamin D supplementation (2000 IU/day) in the first year of life increased the risk of developing atopy, allergic rhinitis, and asthma when assessed at age 31years[66].However, this study was limited by absence of data on maternal intake of vit D and assessment of childhood asthma or atopy.

The observational nature of these studies precludes an assessment of cause and effect. Epidemiological observational studies suggest an association but do not prove causality; and no interventional trials on individuals with low serum vitamin D have evaluated the effect of supplementation on asthma exacerbations, asthma control, or lung function. Such studies are urgently needed since it remains difficult to ascertain from cross-sectional investigations whether vitamin D deficiency is responsible for reduced lung function in asthmatics or whether asthma associated lifestyles, such as less outdoor exercise and thus decreased exposure to sunlight or decreased dietary intake are responsible for lower serum vitamin D levels. More longitudinal and interventional studies in children and adults are needed to elucidate any relationship more clearly. Preventative strategies targeting children are warranted to avoid highly prevalent vitamin D deficiency. Whether advice is need to avoid vitamin D over provision remains more controversial but these results suggest that may be so and improved guidelines regarding vitamin D in childhood are needed, and monitoring of serum vitamin D level may enhance management of respiratory conditions in children.

Conclusion
This cross-sectional study showed that:

Hypovitaminosis D was highly prevalent among Bronchial Asthma (p value < 0.001) as compared to general population.

Bronchial asthma more prevalent in female as compared to male.

Patients age have significant effect on serum vit D levels.

Gender (male/females) have no any predisposing effect on serum vit D levels.

Smoking have no any significant effect on serum vit D levels.

There is no significant difference of serum vit D level in outdoor and indoor population.

Our findings suggest that periodic screening of serum vitamin D and supplementation should be considered in routine care patients with lung diseases. Emphasis should be put on effectiveness of repletion therapy, large sample size and factors that may confound the results, such as; exogenous intake of vitamin D irrespective of the groups assignment, independent effect of nutritional status improvement with therapy and seasonal variation with vitamin D status. The role of vitamin D status in modulating host immune response to respiratory infection and inflammation appears complex. Evidence based information from both clinical and laboratory driven studies are clearly needed to help clarify the complex encircled by vitamin D status, vitamin D metabolism, infection and inflammatory mechanism in human lungs. Given the high frequency of hypovitaminosis D found in this study, there is a need to work more on health

promotion activities to this community targeting the importance of physical activities, exposure to sunlight and use of diets rich in vitamin D.Vit D has a number of activities in addition to its effect on calcium and bone homeostasis and influences process such as immune regulation, host defense, inflammation, or cell proliferation. Several hurdles must be overcome to validate the benefit of Vit D-based therapies: 1) Basic mechanisms are not clear and the involved molecular pathways are likely difficult to identify because Vit D impacts on a variety of biological processes in parallel. 2) Conclusive data from interventional studies are missing for many disease entities. 3) Since Vit D has been used for many years, the pharmaceutical industry might hesitate in starting a development program. Nevertheless, the data available indicate that Vit D could be beneficial for the prevention or therapy of important lung diseases.

References
1. Gomez AC, Naves DM, Rodriguez GM, Fernandez Martin JL, Cannata Andia JB. [Review of the concept of vitamin D "sufficiency and insufficiency"]. *Nefrologia* %2003 ;23Suppl 2:73 -7 2003.
2. Food and Nutrition Board. Institute of Medicine. Dietary reference intakes: water, potassium, sodium, chloride, and sulfate. Washington, DC: *National Academy Press;* 2004.
3. Lappe 1M, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am j Clin Nutr* 2007;85:1586-91.
4. Aloia lF, Talwar SA, Pollack S, Yeh 1. A randomized controlled trial of vitamin D3 supplementation in African American women. *Arch Intern Med* 2005;165:1618-23.
5. Michos ED, Streeten EA, Ryan KA, et al. Serum 25-hydroxyvitamin d levels are not associated with subclinical vascular disease or C-reactive protein in the old order amish. *Calcif Tissue Int.* Mar 2009;84(3):195-202.
6. Costello RB. Vitamin D and health in the 21st century: federal initiatives to advance research. *Am J Med Sci.* Jul 2009;338(1):34-39.
7. Gropper SS, Smith lL, Groff lL. The fat-soluble vitamins: vitamin D. In: AnonymousAdvanced Nutrition and Human Metabolism. Fourth Edition ed. Belmont,CA: Thomson Wadsworth; 2005. p. 343-52.
8. Holick MF. Chapter 18: Vitamin D. In: Shils ME, Olson JA, Shike M, Ross AC, editors. Modem nutrition in health and disease. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 1951,329-345.
9. Omdahl JL, Morris HA, May BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. *Annu Rev Nutr* 2002;22:139.
10. Hilpert J, Wogensen L, Thykjaer T, et al. Expression profiling confirms the role of endocytic receptor megalin in renal vitamin D3 metabolism. *Kidney Int 2002;62: 1672-81.*
11. Dusso AS. Vitamin D receptor: Mechanisms for vitamin D resistance in renal failure. Kidney International Supplement 2003:6-9.
12. Norman A W, Ishizuka S, Okamura WHo Ligands for the vitamin D endocrine system:different shapes function as agonists and antagonists for genomic and rapid response receptors or as a ligand for the plasma vitamin D binding protein. *J Steroid Biochem Mol Bioi* 2001 ;76:49-59.
13. Caballero B, Allen L, Prentice A. Encyclopedia of human nutrition. 2nd ed. Amsterdam Netherlands;; Boston: *Elsevier/Academic Press;* 2005.
14. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? *Brit J Nut* 2003;89:552-72.
15. Omdahl JL, Morris HA, May BK. Hydroxylase enzymes of the vitamin D pathway: expression, function, and regulation. *Annu Rev Nutr* 2002;22:139.
16. Norman A W, Ishizuka S, Okamura WHo Ligands for the vitamin D endocrine system: different shapes function as agonists and antagonists for genomic and rapid response receptors or as a ligand for the plasma vitamin D binding protein. *J Steroid Biochem Mol Bioi* 2001 ;76:49-59.
17. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* 2007;7:684-700.
18. Kimball S, Fuleihan GEH, Vieth R. Vitamin D: a growing perspective. *Crit Rev Clin Lab Sci* 2008;45:339-414.
19. Uitterlinden AG, Ralston SH, Brandi ML, et al. The association between common vitamin D receptor gene variations and osteoporosis: a participant-level meta-analysis. *Ann Intern Med* 2006;145:255-64.
20. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab* 2008;4:80-90.
21. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. Sixth edition ed. Philadelphia: *Saunders Elsevier*; 2007.
22. Ma J, Chen T, Mandelin J, et al. Regulation of macrophage activation. *Cell Mol Life Sci* 2003;60:2334-46.
23. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004; 173 :2909-12.
24. Borderi M et al. Prevalence of hypovitaminosis D among HIV+ patients enrolled in a large Italian cohort. 17th *CROI*, 2010. Poster 75
25. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide(CAMP) gene is a direct target ofthe vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *FASEB J* 2005;19:1067-77.
26. D'Ambrosio D, Cippitelli M, Cocciolo MG, et al. Inhibition ofIL-12 production by 1 ,25-dihydroxyvitamin D3: involvement of NF-kappaB down-regulation in transcriptional repression of the p40 gene. *J Clin Invest* 1998; 1 01 :252-62.
27. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HFJ, O'Garra A. 1,25-dihydroxyvitamin D3 has a direct effect on naive CD4+ T cells to enhance the development ofTh2 cells. *J ImmunoI*2001;167:4974-80.
28. Hewison M, Zehnder D, Chakraverty R, et al. Vitamin D and barrier function: a novel role for extra-renal 1_-hydroxylase. *Mol Cell Endocrinol.*

2004;215(1):31-8.

29. Sears MR. The definition and diagnosis of asthma. *Allergy*. 1993;48(17 Suppl):12–16; discussion 22–23.
30. Holgate ST. Pathogenesis of Asthma. *Clin Exp Allergy*. 2008;38(6):872–97.
31. National Asthma Education and Prevention Program TEP on the D and M of A. Expert panel report 3: Guidelines for the diagnosis and management of asthma [Internet]. U.S. department of Health and Human Services; 2007 Aug. Available from: http://www.ncbi.nlm.nih.gov/books/NBK7223/.
32. Hansdottir, S. and Monick, M. M. (2011) Vitamin D effects on lung immunity and respiratory diseases. *Vitam Horm* 86, 217-37.
33. Lacy, P. and Moqbel, R. (2001) Immune effector functions of eosinophils in allergic airway inflammation. Current opinion in allergy and clinical immunology 1, 79-84.
34. Holgate, S. T. (2008) Pathogenesis of asthma. *Clin Exp Allergy* 38, 872-97.
35. Wegmann, M. (2011) Targeting eosinophil biology in asthma therapy. *Am J Respir Cell Mol Biol* 45, 667-74.
36. Castro, M., Mathur, S., Hargreave, F., Boulet, L. P., Xie, F., Young, J., Wilkins, H. J.,Henkel, T., Nair, P. (2011) Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med* 184, 1125-32.
37. Haldar, P., Brightling, C. E., Hargadon, B., Gupta, S., Monteiro, W., Sousa, A., Marshall, R. P., Bradding, P., Green, R. H., Wardlaw, A. J., Pavord, I. D. (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360, 973-84.
38. Rodrigo GJ, Rodrigo C, Hall JB. Acute asthma in adults*: A review. *CHEST J*. 2004 Mar 1;125(3):1081–102.
39. Masoli M, Fabian D, Holt S, Beasley R, Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004 May;59(5):469–78.
40. Garcia-Aymerich J, Varraso R, Danaei G, Carlos A. Camargo, Hernán MA. Incidence of Adult-onset Asthma After Hypothetical Interventions on Body Mass Index and Physical Activity: An Application of the Parametric G-Formula. *Am J Epidemiol*. 2013 Oct 9;kwt229.
41. Litonjua AA. Dietary Factors and the Development of Asthma. *Immunol Allergy* Clin North Am. 2008 Aug;28(3):603–ix.
42. Raby BA, Lazarus R, Silverman EK, et al. Association of Vitamin D Receptor Gene Polymorphisms with Childhood and Adult Asthma. *Am J Respir Crit Care Med*. November 15; 2004 170(10):1057–1065. [PubMed: 15282200].
43. Bosse Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. *Physiol Genomics*. Apr 24; 2007 29(2):161–168.
44. Poon AH, Laprise C, Lemire M, et al. Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med*. Nov 1; 2004 170(9):967–973.
45. Banerjee A, Damera G, Bhandare R, et al. Vitamin D and glucocorticoids differentially modulate chemokine expression in human airway smooth muscle cells. *Br J Pharmacol*. Sep; 2008 155(1): 84–92.
46. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. *J Immunol*. Jan 15; 184(2):965–974.
47. Bosse Y, Maghni K, Hudson TJ. 1alpha,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. *Physiol* Genomics. Apr 24;2007 29(2):161–168.
48. Song Y, Qi H, Wu C. Effect of 1,25-(OH)2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. *Respirology*. Jul; 2007 12(4):486-494.
49. Afzal S, Lange P, Bojesen SE, Freiberg JJ, Nordestgaard BG. Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease. *Thorax*. 2013 Aug 1; thoraxjnl–2013–203682.
50. Plum LA, DeLuca HF. Vitamin D, disease and therapeutic opportunities. *Nat Rev Drug Discov*. 2010 Dec;9(12):941–55.
51. Gupta A, Sjoukes A, Richards D, Banya W, Hawrylowicz C, Bush A, et al. Relationship between Serum Vitamin D, Disease Severity, and Airway Remodeling in Children with Asthma. *Am J Respir Crit Care Med*. 2011 Dec 15;184(12):1342–9.
52. Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DYM. Vitamin D Levels, Lung Function, and Steroid Response in Adult Asthma. *Am J Respir Crit Care Med*. 2010 Apr;181(7):699–704.
53. Alyasin S, Momen T, Kashef S, Alipour A, Amin R. The Relationship Between Serum 25 Hydroxy Vitamin D Levels and Asthma in Children. *Allergy Asthma Immunol* Res. 2011 Oct;3(4):251–5.
54. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y, Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. *Am J Clin Nutr*. 2010 May 1;91(5):1255–60.
55. Lewis E, Fernandez C, Nella A, Hopp R, Gallagher JC, Casale TB. Relationship of 25-hydroxyvitamin D and asthma control in children. *Ann Allergy Asthma Immunol*. 2012 Apr;108(4):281–2.
56. Algert CS, Bowen JR, Lain SL, Allen HD, Vivian-Taylor JM, Roberts CL. Pregnancy exposures and risk of childhood asthma admission in a population birth cohort. *Pediatr Allergy Immunol*. 2011;22(8):836–42.
57. Bäck O, Blomquist HKS, Hernell O, Stenberg B. Does vitamin D intake during infancy promote the development of atopic allergy? *Acta Derm Venereol*. 2009;89(1):28–32.
58. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120:1031–5.
59. Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey*. *CHEST J*. 2005 Dec 1;128(6):3792–8.
60. Berry DJ, Hesketh K, Power C, Hyppönen E. Vitamin D status has a linear association with seasonal infections and lung function in British adults. *Br J Nutr*. 2011;106(09):1433–40.
61. Afzal S, Lange P, Bojesen SE, Freiberg JJ, Nordestgaard BG. Plasma 25-hydroxyvitamin D, lung function and risk of chronic obstructive pulmonary disease. *Thorax*. 2013 Aug 1; thoraxjnl–2013–203682.
62. Zarogoulidis P, Porpodis K, Domvri K, Milonaki E, Primikiri S, Gouleka A, et al. Vitamin D in asthma and future perspectives. *Drug Des Devel Ther*. 2013 Sep;1003.
63. Searing DA, Zhang Y, Murphy JR, et al. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *J Allergy Clin Immunol* 2010;125:995–1000.
64. Brehm JM, Schuemann B, Fuhlbrigge AL, et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol* 2010.
65. Wjst M. The vitamin D slant on allergy. *Pediatric Allergy and Immunology* 2006;17:477–83.
66. Hyppo¨nen E, Sovio U, Wjst M, et al. Infant Vitamin D Supplementation and Allergic Conditions in Adulthood: Northern Finland Birth Cohort 1966. *Annals of the New York Academy of Sciences* 2004;1037:84–95