SERUM 25 OH VITAMIN D IN CHILDREN WITH BACTERIAL PNEUMONIA

Wagdy A. El-sayed, and Eman R. Aamer
Department of Pediatric and Clinical Pathology; Banha Teaching Hospital

ABSTRACT

Background: Bacterial pneumonia of is common cause of pediatric mortality and hospital admission. Different risk factors for pneumonia are there. One possible risk factor is vitamin D deficiency .**Objective:** We determined 25 OH-vitamin D in bacterial pneumonia.to evaluate whether its level has relation to pathogenesis and management of the disease **Design**: case-control study **Methods:** 25 OH-vitamin D was determined by competitive enzyme immunoassay (EIA) commercially available kit in 36 cases with pneumonia CRP was determined by ELIZA "high sensitive CRP" .ESR done by Wintrobe method The results were analyzed and compared with 16 controls using SPSS 20 **Results**: Vitamin D level in pneumonia cases was significantly lower in patients compared to controls and showed negative correlation with C-reactive protein and weak negative correlation ESR. Vitamin D did not show any relation to age, sex, weight or other clinical symptoms as dyspnea and fever Also did not show any relation to RBCs, Hb, or WBCs .**Conclusion**: We conclude that level of 25 OH-vitamin D is low in children with bacterial pneumonia and correlates negatively with CRP with possible relation to disease severity.

Keywords: pneumonia, 25OH vitamin D, bacterial and viral infection

INTRODUCTION

Pneumonia is the leading cause of childhood mortality, accounting for 19% of the 10.6 million deaths that occur each year worldwide **(1)**. Accumulating evidences about relation of vitamin D to immunity developed through many observations.

Children with rickets are more likely to develop pneumonia or tuberculosis than children without rickets. **(2)**Deficiency or insufficiency of vitamin D was associated with increased risk of infection after dental procedures and other types of infections **(3)** Also some studies suggested that vitamin D and its metabolites operate at tissue, cellular, and nuclear sites and likely alter immune function at a subcellular level**(4).**

Vitamin D deficiency is more increasing in frequency and distribution even in sunny countries as India , Nepal **(5)** and Bangladesh **(6).** Among U.S. children from 1-11 years 18 % has 25 OH vitamin D < 50 nmol/l " deficiency " 69 % has level < 75nmol /l "insufficiency" **(7).**

The relation of vitamin D deficiency to respiratory tract infection has been evaluated in some reports. Lower serum 25 (OH) D the circulating marker of vitamin D status levels are associated with increased risk of upper **(8)** as well as lower respiratory tract infection **(9, 10).**

Subclinical vitamin D deficiency has been also associated with an increased risk of tuberculosis in adults through modification of polymorphisms in the vitamin D receptor (VDR) **(11).**

Most reports about vitamin D in pneumonia was described in adults **(12 & 13),** very few reports available in children (**14).**

In our localities there is possibility of vitamin D deficiency is present and evidenced due poor housing in urban and malnutrition in rural area. It is possible that vitamin D deficiency is important risk factors for pneumonia in some populations. For these reasons , we determined 25-OH vitamin D in cases proven to be bacterial pneumonia to highlight possible anti-infectious and immunological role of vitamin D in this disease with possible role in management and prevention.

SUBJECTS & METHODS

Patients group: 36 children diagnosed as bacterial pneumonia with age ranged from 2.5 to 9 years were included in the study. Detailed medical history and clinical examination was done for each case.

Definition of pneumonia. Pneumonia *was* ***defined*** as "*case definition*" temperature greater than 38 ^{o}C associated with consolidation on chest radiograph (interpreted independently by two physicians), and tachypnea (respiratory rate of 60 breaths per minute for children aged 0–2 months, 50 breaths per minute for children aged 3–12 months, and 40 breaths per minute for children aged 13–60 months)**(15).**

Exclusion criteria for patients: Children with pronounced wheezes or those who had clinical signs of rickets or were known to have received high-dose vitamin D treatment in the past 3 months were excluded from the study. Patients with history of preterm delivery and NICU admission, those with hepatic or renal disorders, measles, severe malnutrition and suspected tuberculosis were also excluded .Also very critically ill cases which required ICU admission

were not included because of small number and difficult sampling.

The control group: were selected from 1ry health care center coming for routine care as immunization, minor illness not related to infection, allergy or bone development. Those with history suggest respiratory illness in last one month or hospital admissions for pneumonia in last one year ***were not included*** **(16).** CBCs, CRP, ESR**,** blood culture for patients as well as controls.

Determination of 25-OH vitamin D:3 ml of venous blood sample were drown under complete aseptic precaution from patients & controls , putted into vacationer serum tubes ,then allowed to clot followed by centrifugation with subsequent prompt separation of serum. Samples precipitated with precipitation reagent to attract 25-OH vitamin D binding protein .The supernatant of samples collected, stored at -20 ^{O}C for subsequent assay.

Principle of measurement of 25-OH vitamin D:

25 OH-vitamin D was measured by competitive enzyme immunoassay (EIA) by commercially available kit from Immune Diagnostic Company from Australia. The procedure followed the basic principle of competition enzyme immunoassay where there is a competition between an unlabeled antigen (vitamin D in the sample) and enzyme-labeled antigen to bind to the antibody binding sites on the wells. The amount of enzyme-labeled antigen bound to the antibody is inversely proportional to the concentration of the unlabeled analyte present in the sample. Unbound material was removed by decanting and washing the wells. Quantitation of enzyme labeled antigen is achieved by incubation with a host specific peroxidase antibody using TMB (tetra methyl Benzedrine) as enzyme substrate .The color converted to yellow. The intensity of the color is indirectly proportional to concentration of 25 -OH vitamin D in the sample (**17)**. A set of 25-OH vitamin D standards were used to plot a standard curve of absorbance versus 25-OH vitamin D concentration from which 25-OH vitamin D concentration was calculated

CRP was determined by ELIZA "high sensitive CRP". ***ESR*** done by Wintrobe method

Legal aspect: Written consent has been taken from parents of all children in this study. The study also was approved from research ethics committee in General Organization For Teaching Hospital and Institutes in Cairo

Statistical Analysis**:** Data were entered and analyzed by using *SPSS 20*.Data were expressed as mean ±SD for categorized variables. Test of significance "Chi-square and student T tests" and correlation study done when appropriate. P < 0.05 was statistical significant.

RESULTS

No significant differences between cases controls regarding age, sex and weight (P was 0.598, 0.105 and 0.15 respectively) **table 1**. No sex difference in vitamin D level among the cases (P; 0.219), **table 2.**

Characters of patients group : Among pneumonia cases 20 (55.6%) had moderate respiratory distress, While 16 (44.4 %) had severe distress. Also 15(41.6%) cases had cyanosis, 19 (52.8%) cases had normal color. Two cases (5.6%) had pallor. 31cases (86.1) had fever, 5 cases (13.9 %) did not had fever.

Vitamin D regarding clinical signs: No difference in vitamin D level between cases showed moderate and that with severe distress , cases showed normal color and that with cyanosis or cases developed and that not developed fever (P was 0.485 , 0.087and 0.441 respectively),**table 2**.

Laboratory parameters in patients and controls: Hb, Htc, RBCs and compared to control (P was 0.000, 0.000 and 0.036 respectively), while showed higher level of inflammatory marker including WBCs, CRP and ESR (P was 0.000 for the three parameter). However, no significant difference was found regarding platelet count P was (0.289) **(Table 3)**. Only 7 cases (19.44%) were found to have +ve blood cultures.

Vitamin D in patients versus controls: ***Pneumonia*** cases showed lower vitamin D compared to controls, "P; 0.000 " **(Table 3 ,figure 1**).With stratification 20 (55.6 %) cases showed level between 50-80nmol/l "insufficiencies" 2 cases (5.6 %) showed level < 50 nmol/l "deficiency " while 14 cases (38.9 %) showed level between 80-250 nmol/l "sufficiency" **figure 2** .No cases showed level above 250 nmol/l "excess " **(18)** . All controls were in range of sufficiency .Nothing of them were in range of insufficiency, deficiency or excess.

Vitamin D relation to laboratory parameters :Vitamin D level did not show any relation to Hb ,RBCs , Platelets , WBCs, age or sex (P was 0.108 ,0.484, 0648 , 0.513, 0.247 and 0.307 respectively) .However ,weak non-significant correlation was found between vitamin D level and ESR (P was 0.052), while strong negative correlation was found between vitamin D level and CRP , P was 0.000 (**table 4 ,figure 3**).

Table (1) demographic data

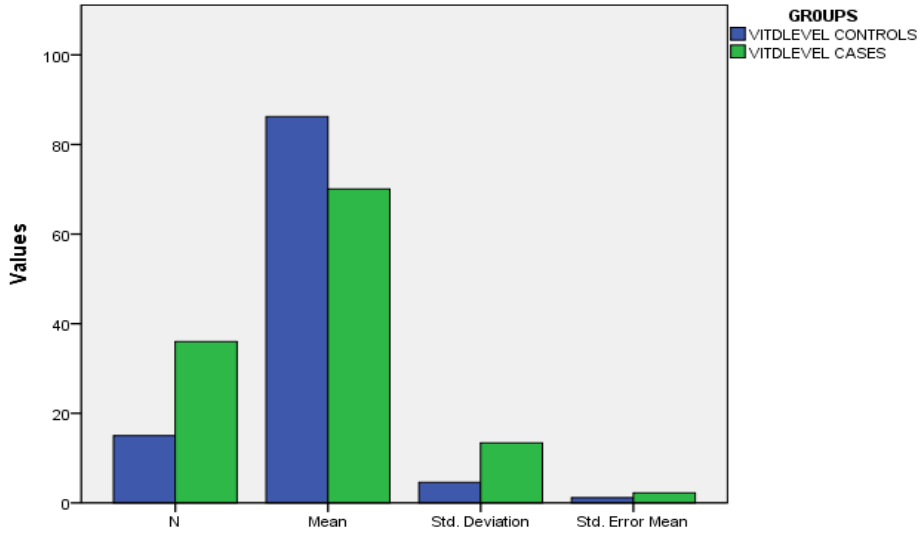
		Controls n=16	Cases n=36	p
Age(ys)	Ẍ ±SD	5.63± 1.99	5.3±2	0.598 NS
	Range	3-9	2.5- 9	
Sex	♀	11- 68.8%	16- 44.4%	0.105 NS
	♂	5- 31.2%	20- 55.6%	
Wt.(kg)	Ẍ ±SD	17.786±4.42	19.527± 3.745	0.15 NS
	Range	12-28	13-26	

Table (2) Vitamin D Regarding Clinical Data

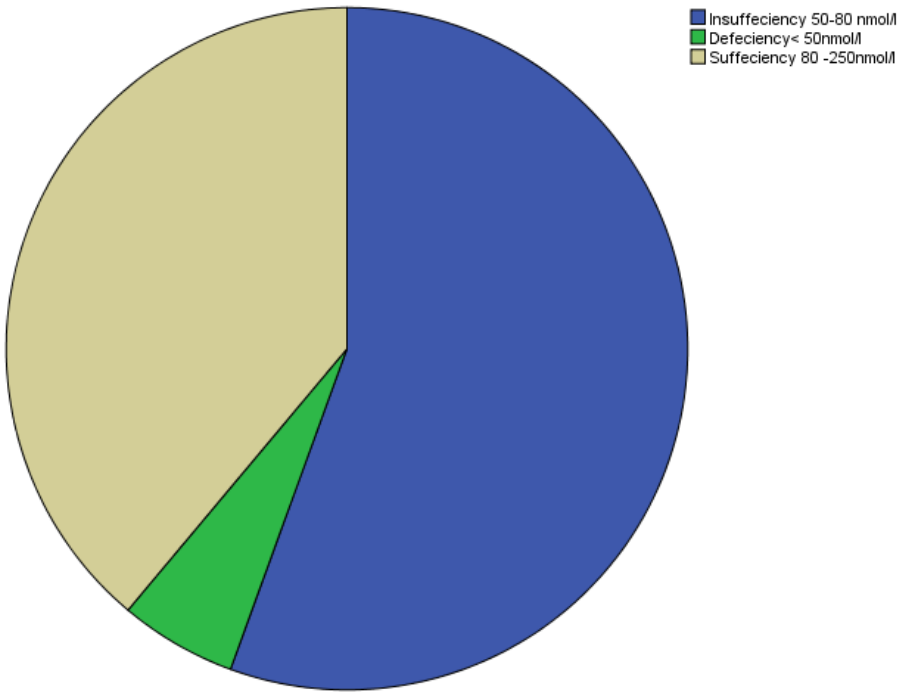
			Ẍ ±SD	P
Sex	♀ n=16	44.4%	73.178±12.723	0.219 NS
	♂ n=20	55.6%	67.6±13.739	
Dyspnea	Moderate n=20	55.6%	67.88±11.596	0.485 NS
	Severe n=16	44.4%	71.312±16.069	
Fever	+ve n=30	86.1%	69.3±13.643	0.441 NS
	-ve n=6	13.9%	74±12.522	
Color	Normal N=19	52.8%	74.117±10.746	0.087 NS
	Cyanosis N=15	41.6%	66.666±13.042	

Table (3) Cases versus Control Regarding Laboratory Data

		Control=16	Cases=36	P
Hb (gm/dl)	Ẍ ±SD	12.83±0.7	11.31±1.112	0.000 HS
	Range	11-12.83	9.5-12.8	
Htc	Ẍ ±SD	36.62±2.187	32.277±3.738	0.000 HS
	Range	33.00-40.00	26.00-39.00	
RBCs(×106)	Ẍ ±SD	4.306±0.429	4.006±0.47	0.036 S
	Range	3.50-5.10	3.20-4.900	
WBCs	Ẍ ±SD	6910±18789.368	12571.111±2356.585	0.000 HS
	Range	4200.00-11000.00	5500.-8700.00	
CRP(mg/dl)	Ẍ ±SD	5.75±3.	35±30.544	0.000 HS
	Range	1-12	12-96.00	
ESR1st hour	Ẍ ±SD	8.06±2.54	19.86±6.7	0.000 HS
	Range	4-13	10.00-40.00	
ESR2nd hour	Ẍ ±SD	18.94	38.75±12.89	0.000 HS
	Range	15-22	15.00-70.00	
Platelets	Ẍ ±SD	380.625±95.182	342.5±155.79	0.289 NS
	Range	250.00-550.000	110.00-650.00	
Vitamin D Level	Ẍ ±SD	86.287±4.43	70.083±13.408	0.000 HS
	Range	80.1-95	40-98	



Vitamin in Patients versus Controls
Figure 1



Stratified Vitamin D Level among Cases
Figure 2

(4) Vitamin D Regarding Laboratory Data, age and sex

	R	P
Hb	0.112	0.108 NS
CRP	0.820	0.000 HS
RBCs	-0.131	0.484 NS
WBCs	-0.113	0.513 NS
PLATLET	0.079	0.648 NS
ESR	-0.326	0.052 NS
Age	0.198	0.247 NS
Wt.	0.175	0.307 NS

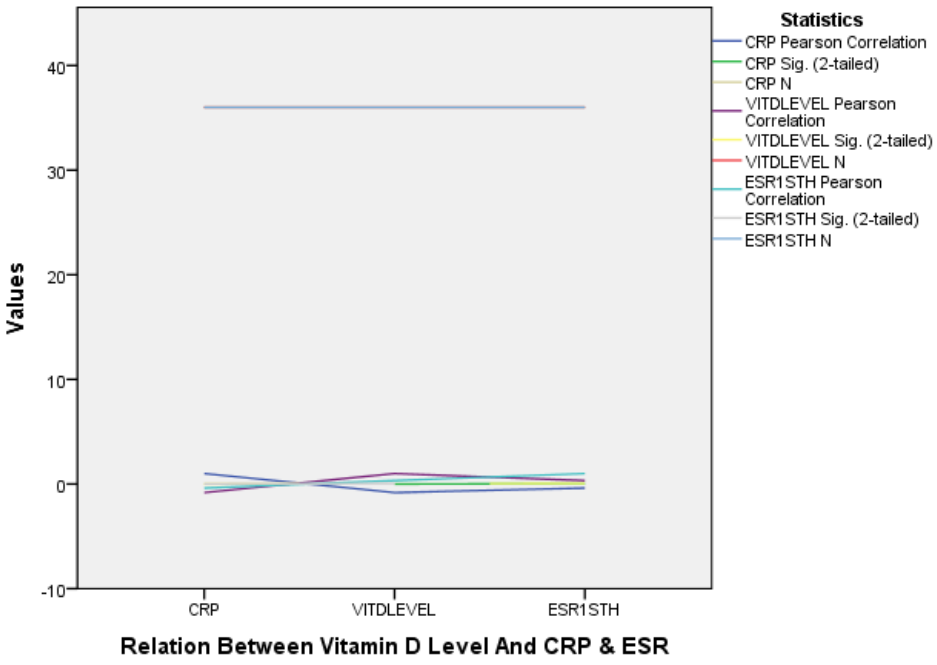


Figure 3

DISCUSSION

In this study we determined 25 OH-vitamin D in 36 cases with bacterial pneumonia, age .sex and weight matched with 16 healthy control (P was 0.598, 0.105 and 0.15 respectively).

We measured 25-OH vitamin D rather than 1,25 vitamin D because it is reliable measure for vitamin D .It reflects the store of vitamin D, it is bounded to vitamin binding protein (VDBP) , it constitutes the major circulating form . The active form 1, 25 OH -vitamin D occurs locally in renal and extra renal tissues. The local production of 1,25 (OH)2D3 by cells and the ability for the hormone to act directly on surrounding tissues, emphasizes the possibility that circulating plasma levels of 1,25(OH)2D3 may not truly reflect what is going on in specific tissues and may be variable **(19)**.

The aim of this study to investigate possible anti infectious and immunological role of vitamin D, that possible vitamin D deficiency may be a risk factor for this disease which might be reflected on its management and prevention.

We excluded cases with possible underlying diseases which can cause pneumonia with possible associated disturbance in vitamin D metabolism such as rickets, malnutrition and those

with previous preterm delivery or admission in NICU (**17)**. Critically ill cases and cases required admission to ICU were statistically insignificant and was excluded from the study. Cases and control were matched in age, sex and weight.

Pneumonia cases showed significant lower Hb ,Htc ,RBCs (P was 0.000 ,0.000 ,0.036 respectively) , higher WBCs ,CRP , ESR count (P was 0.000) compared to control . These finding is expected because of associated inflammation and infection in pneumonia of bacterial origin**.** Only 7cases (19.44%) showed positive blood cultures. Blood culture is positive only in 10-30 % of bacterial pneumonia **(20)**

Regarding 25 OH vitamin D , it was found significantly lower in pneumonia cases in relation to control (P was 0.000) with significant negative correlation with CRP , non-significant negative correlation with ESR (P was 0.058) . 20(55.6 %of cases were in range of insufficiency, 2 cases (5.6 %) in range of deficiency, 14 (38.9%) in range of sufficiency. All controls were in range of sufficiency. No relation was found to clinical or laboratory signs.

These findings are consistent with epidemiological studies of increased respiratory tract infection in winter time with decreased vitamin D synthesis due to decreased exposure to UV B radiation **(21)** and higher rates of ALRI in children with vitamin D deficient rickets **(22)**

In agreement with our results , **Roth DE et al 2010 (17)** from Bangladesh found significant lower serum 25(OH) vitamin D among acute lower reparatory tract infection (ALRI) cases (29.1 nmol ⁄ L, SD 17.2) compared with matched controls (39.1 nmol⁄L SD 9.4); the mean case–control difference was 10.0 nmol ⁄L (standard error, 3.68 nmol ⁄ L; p = 0.0146). **Ways V, et al 2004(9)** found that subclinical 25(OH) vitamin D deficiency at 4 month is a risk factor for developing ALRI in Indian children under 5 years In Turkey **Karatekin G, 2009 et al (10)** found association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers.

These previous studies done in lower respiratory tract infection in children with similar sunny atmosphere and social classes average and below average, however they give lower values of mean vitamin D relative to our study. Our higher values can be explained by higher age range of cases beyond infancy with more sun exposure and little affected by maternal vitamin D. **Ways V, et al 2004(9)** found that serum 25-OH vitamin D increased significantly with age in both cases and controls. Also, these studies included pneumonia of viral and bacterial origin in addition to bronchiolitis. **Jing R** et al **(14)** found lower vitamin D in severe community acquired pneumonia (CAP) of bacterial etiology in children compared with control and mild cases. Also **Pletz M W et al 2014 (12)** found that vitamin D correlates negatively with disease severity independent of age, season, pathogen in adults. **Jovanovich A J. et al 2014** found that vitamin D deficiency is associated with risk of CAP and sepsis in adults (**13)**

On the other hand, in one Canadian study done by **McNally D J et al (23)**, no significant difference was found between cases with ALRTI and control this study included pneumonia and bronchiolitis however in this study the pneumonia subgroup showed a trend towards a lower unadjusted mean vitamin D and a higher proportion of participants with vitamin D deficiency but still non-significant after adjustment. Cases which admitted in ICU had significant lower 25 OH vitamin D compared with ward admission. Our values for vitamin D in controls are similar to these last study , but our cases had lower values . A possible explanation for these differences in case selection with different disease severity. It is possible that our study included more severe cases more than that included in the previous study done by **McNally D J et al.** His severe cases admitted to ICU showed lower level of vitamin D in relation to control. The criteria for ICU admission may vary from unit to another according to availability of staff and other facilities. Our finding of negative correlation between vitamin D and inflammatory markers support this explanation. In adults ,one study in critical care setting found that decrease in circulating C reactive protein and IL-6 with high dose vitamin D (500 IU vs. 200 IU) **(24)** this support possible relation of vitamin D to disease severity . **McNally D J** explained variable results regarding vitamin D in ALRTI in literatures due to variable severity of the disease in different reports **(23)**

A possible explanation of association of vitamin D deficiency in pneumonia that vitamin D influences antimicrobial activity, inflammation, and coagulation partly by regulating calcium and phosphorous homeostasis and by acting on lymphocytes, neutrophils, macrophages, and respiratory epithelial cells (**25**).The endogenous synthesis of a host antimicrobial peptide (LL- 37) is stimulated by the activation of the vitamin D receptor by 1,25-OH vitamin D in monocytes **(26)** and respiratory epithelial cells **(27).** Up regulation of, LL-37 in tracheal secretions during lower

respiratory tract infections in infants further suggests its potential role in innate defenses against ALRI **(28)**

Some limitation to our study may be small control size but results are powered by statistical analysis "independent sample t test". We did not include cases required ICU admission in the study because of small number and insignificant statistically.

Further studies required to confirm our results with larger number of cases with different severity including ICU admissions in different type of pneumonia in different populations with clinical and subclinical vitamin D deficiency to verify whether these results are due to respiratory or anti infection function.

Our results have clinical implication. Vitamin D is considered micronutrient, its deficiency is a common health problem in most communities fortification or supplementation may have beneficial effect in pneumonia cases which is common also especially with clinical or laboratory of vitamin D deficiency. In area with evidenced deficiency of vitamin D deficiency trial of vitamin D supplement with single dose of 100.000 i.u. did not improve reduce duration of recovery but reduced number of recurrent episodes in subsequent 3 months without any side effects **(29)** On other hand short term supplement with oral vitamin D (1000-2000 IU per day for 5 days) has no beneficial effect on resolution of severe pneumonia in under-five year age **(30)** This trial needs also further evaluation for identification of selected patients and adjustment of beneficial dose.

CONCLUSION

We determined 25 OH vitamin D in cases with bacterial pneumonia we found significant lower vitamin D in pneumonia cases compared to control cases with high significant negative correlation with CRP, weak negative correlation with ESR with no relation to other clinical or laboratory parameters .These results may has implication on prevention and management of bacterial pneumonia.

REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K & Black RE (2005)WHO estimates of the causes of death in children. Lancet: 365, 1147–1152.
2. Holick MF. (2007) Vitamin D deficiency. N Engl J Med; 357:266-81
3. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. (2008) Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics; 122: 398-417.
4. Bouillon R, Bischoff-Ferrari H, Willett W. (2008) Vitamin D and health: perspectives from mice and man. J Bone Miner Res; 23:974-979.
5. Jiang T, Christian P, Khatry SK, Wu L, West KP Jr. (2005) Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. J Nutr; 135: 1106–1112.
6. Islam MZ, Lamberg-Allardt C, Karkkainen M, Outila T, Salamatullah Q, Shamim AA. (2002) Vitamin D deficiency: a concern in premenopausal Bangladeshi women of two socio-economic groups in rural and urban region. Eur J Clin Nutr; 56: 51–56
7. Mansbach J M., Ginde Adit A, Camargo Jr. and Carlos A., (2009)Serum 25-Hydroxyvitamin D Levels Among US Children Aged 1 to 11 Years: Do Children Need More Vitamin D? Pediatrics November; 124(5): 1404–1410
8. Ginde, A.A.; Mansbach, H.M.; Camargo, C.A., Jr. (2009) Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch. Intern. Med., 169, 384–390.
9. Wayse V, Yousafzai A, Mogale K, Filteau S. (2004) Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. Eur J Clin Nutr; 58: 563–567.
10. Karatekin G, Kaya A, Salihog˘lu O, Balci H, Nuhog˘lu (2009) A. Association of subclinical vitamin D deficiency in newborns with acute lower respiratory infection and their mothers. Eur J Clin Nutr; 63: 473–477.
11. Wilkinson RJ, Llewelyn M, Toossi Z et al. (2000) Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case control study. Lancet , 355: 618–621.
12. Pletz M W, Terkamp C, Schumacher U, Rohde G, Schütte H, Welte T and Bals R (2014) Vitamin D deficiency in community-acquired pneumonia: low levels of 1,25(OH)2 D are associated with disease severity . Respiratory Research, 15:53-61
13. Jovanovich A J, Ginde A A, Holmen J, Jablonski K, Allyn R L, K J and Chonchol M (2014) Vitamin D Level and Risk of Community-AcquiredPneumonia and Sepsis Nutrients, 6, 2196-2205
14. Jing R, Bin S, Po M, and King F, (2013) Correlation between serum vitamin D level and severity of community acquired pneumonia in young children [Chin. J. Contemp. Pediatr., , 15(7): 519-521
15. Jadavji T, Law B, Lebel MH, Kennedy WA, Gold R and Wang EE(1997): A practical guide for the diagnosis and treatment of pediatric pneumonia. CMAJ; 156:S703–S711.
16. Roth DE, Shah R, Black RE and Baqui AH (2010)Vitamin D status and acute lower respiratory

infection in early childhood in Sylhet, Bangladesh Acta Paediatrica 99, 389–393

17. Wicherts IS, van schoor NM, Borke AJ, visser M, Deeg Di, smit J Knol DL and Lips P(2007): Vitamin D status predicts physical performance and its decline in older person J. Clin. Endocrinol. Metab., Jun., 92 (6): 2058-2065
18. Grant W B, and Holick M F, (2005) Benefits and Requirements of Vitamin D for Optimal Health: A Review Alternative Medicine Review Volume 10, Number 2 Page 95-111
19. Barnes M S, Robson P J, Bonham M P, Strain and Julie JJ and Wallace MW(2007) Vitamin D: Status, Supplementation and Immunomodulation Current nutrition & Food Science, , 2, 315-336.
20 . Sectish T C. and Prober C G. Pneumonia, In Nelson Textbook of Pediatrics, 19th ed. Copyright © 2011 Chapter 389: 1474-1479, eds.. Kliegman RM, Beherman RE, Jensen HB, Santon BF. Saunders, An Imprint of Elsevier
21 Najada AS, Habashneh MS, and Khader M (2004)The frequency of nutritional rickets among hospitalized infants and its relation to respiratory diseases. J Trop Pediatr 50:364–368.
22. Aloia JF, Li-Ng M. Re (2007): Epidemic influenza and vitamin D Epidemiol Infect; 135: 1095–1096. author reply 1097-1098.
23. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, and Rosenberg AM, (2009) Vitamin D Deficiency in Young Children With Severe Acute Lower Respiratory Infection Pediatr. Pulmonol.; 44:981–988.
24. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. (2003) Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol. Metab.; 88: 4623–4632.
25- Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. (2007) Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). J Cyst Fibros 2007; 6:403–410.
26. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al.(2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science; 311: 1770–1773
27. Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. (2008) Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. J Immunol; 181: 7090–7099.
28-. Schaller-Bals S, Schulze A, Bals R. (2002) Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection. Am J Respir Crit Care Med; 165: 992–5
29- Manaseki-Holland S, Qader G, Masher MI2, Bruce J, M. Mughal MZ, Chandramohan D and Walraven G. (2010) Effects of vitamin D supplementation to children diagnosed with pneumonia in Kabul: a randomized controlled trial; 15 (10) 1148–1155
30. Choudhary N And Gupta P (2012) Vitamin D Supplementation for Severe Pneumonia –A Randomized Controlled Trial Indian Pediatr 49(6):449-554