Original Research Article

Correlation of serum vitamin D levels and anemia in childhood pneumonia: a case control study from rural area

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ABSTRACT

Background: Pneumonia remains number one cause for morbidity and mortality in children. Vitamin D has been shown to have immunomodulatory activity. Hence to find correlation between vitamin D and pneumonia cases the study was carried out.

Methods: A case control study was conducted at a rural hospital in Vadgaon. From September 2015 onwards all children under 5 years of age hospitalized with pneumonia were invited to participate in our study. Patients who consented for study underwent serum vitamin 25 (OH) D3 estimation along with other routine investigations as indicated. During the same study period, parents of otherwise healthy children fewer than 5 years age who came to our immunization clinic were invited to participate in the study as a control group.

Results: Vitamin 25(OH) D3 levels in pneumonia cases study group (N=40) 12.048±6.015 ng/ml (mean±SD) and in control (N=35) 22.65±11.77 ng/ml (mean±SD) that is statistically significant (P<0.0001) and hemoglobin (Hb) in Cases (N=40) with 8.55±1.27 gm/dl (mean±SD); control group (N=19) with 10.31±0.9 gm/dl (mean±SD)levels was found to be statistically significant (p < 0.0001).All patients with severe pneumonia needing ICU management were found to be deficient in vitamin D levels. Children with pneumonia had higher level of anemia. All children admitted to ICU had Moderate to severe anemia.

Conclusions: There is a correlation between Vitamin D deficiency and severity of pneumonia and there is also correlation between level of anemia and severity of pneumonia.

Keywords: Anemia, Lower respiratory tract infection, Pneumonia, Vitamin D deficiency

INTRODUCTION

Pneumonia remains the number one cause for under five deaths worldwide. Out of 6.3 million children dying before 5 years, 0.935 million patients die due to Pneumonia.[1] Different approaches have been tried to reduce the morbidity and mortality due to pneumonia such as immunization, vitamin A supplementation and zinc supplementation.[2]

The possible role of vitamin D in immune-modulation is explored since last 25 years. However, it is only in 2006 that the role of vitamin D in immune function has been confirmed.[3] The vitamin D converts immune response from TH1 to TH2 response. It also helps in rapid response at the cellular level. It helps in activation of macrophages.[4,5] It is also noted that children with pneumonia had lower vitamin D levels.[6-8] They also may have lower dietary vitamin D intake.[9]

Keeping this in mind, we decided to perform an evaluation of vitamin D levels in patients diagnosed to have pneumonia. We also aimed to investigate impact of severity of vitamin D deficiency on severity of pneumonia. We also investigated the prevalence of

anemia in this patient and its impact on severity of pneumonia. Ours is the study from a rural India addressing the possible impact of both vitamin D deficiency and anemia in children hospitalized with pneumonia.

METHODS

The study type was case control study.

Objective of the study was to find out prevalence of 25 (OH) D3 deficiency and anemia in children hospitalized with pneumonia and to study impact of severity of vitamin D deficiency and anemia on need for admission to intensive care unit in children with pneumonia in a rural hospital.

Sample size

A sample size of 40 children per group was needed as per power calculation using [alpha] = 0.05 and [beta] = 0.80.

Settings of study and subjects

From September 2015 onwards all children under 5 years of age hospitalized at rural hospital, Vadgaon and diagnosed with pneumonia were invited to participate in our study. Patients with rickets and associated other systemic diseases were excluded from study. Similarly children who had history of receiving vitamin D supplementation in last 1 year were excluded from study. Infants < 6 months were also excluded from study. All patients in study group underwent a detailed history followed by clinical examination. An x-ray chest was performed in all patients along with complete blood count and other biochemical investigations. Patients were diagnosed as pneumonia by a pediatrician (AC) based on history, examination and chest X-ray findings.[10] The criteria used for diagnosis of pneumonia were:

- History of high-grade fever, cough and breathlessness
- On examination- Respiratory rate more than as described by WHO, chest indrawing, bronchial breathing and crepitations.
- Chest X-ray PA view.

Patients with severe pneumonia were admitted in pediatric intensive care unit (ICU). Criteria for ICU admission were:

- Severe respiratory distress
- Drowsiness
- Oxygen saturation by pulse oximetry < 92%
- Not accepting oral feeding
- Intervention and principal outcome measured

After the diagnosis of pneumonia all patients who consented for study underwent serum vitamin 25(OH) D3 estimation by CLIA method. Criteria used to define vitamin D deficiency are as follows.[11]

- Vitamin D deficiency: <20 ng/ml
- Vitamin D insufficiency: 20-30 ng/ml
- Vitamin d sufficiency: 30-150 ng/ml
- Vitamin D toxicity: > 150 ng/ml

Similarly children with hemoglobin level (Hb) less than 11gm/dl were considered to be anemic. They were further classified as mild anemia (Hb-10 to10.9 mg/dl); moderate anemia (Hb -7 to 9.9 gm/dl) and severe anemia (Hb <7 gm/dl).[12]

During the same study period, parents of otherwise healthy children fewer than 5 years age who came to our immunization clinic were invited to participate in the study as a control group. After obtaining the consent and administration of immunization, the blood sample of child was collected for serum vitamin 25 (OH) D3 estimation. The parents were informed that if incidental vitamin D deficiency is detected he would be appropriately treated for it by vitamin D supplementation.

Statistical analysis

All the measurable data i.e. quantitative variables were expressed in terms of their mean and SD and category variables in terms of proportion. For comparison of means unpaired t-test was used and for comparing of proportion Z-test was used. P value <0.05 was considered statistically significant. To see association of group's Chi-square test or Fisher's exact test is used whenever applicable. Descriptive statistics was calculated by using MS-Excel 2007 and for inferential statistics Graphpad Quick cal Instat Software was used.

Ethical issues

Parents of the cases and controls were informed about the study. The blood was collected after informed consent. The cost of investigation was borne by the investigators. Hospital ethical committee approved this study.

RESULTS

Forty patients were enrolled as planned in the study from September 2015 to January 2016. During the same period 54 children between 6 months and 5 yrs of age came to our immunization clinic. Of this 35 consented to participate as controls in the study. Parents of remaining 19 children's did not consent to participate in the study. Patients demographic data recorded were age, sex, height, weight and mid arm circumference (MAC). Patient's age and weight were comparable in both groups (Table 1). We noted that study group had more female population and were having lower height and MAC. The difference in these parameters was found to be statistically significant (p <0.05). We found that children with pneumonia had lower Vitamin 25 (OH) D level study group (N=40)12.048±6.015ng/ml (Mean±SD) and in control (N=35) 22.65±11.77ng/ml (mean±SD) that is statistically significant (P <0.0001) and hemoglobin (Hb)

in Cases (N=40) with 8.55±1.27gm/dl (mean±SD); control group (N=19) with 10.31±0.9gm/dl (mean±SD) levels was found to be statistically significant (p <0.0001).

Table 1: Patients demographic data.

	Study group (N=40) (mean±SD)	Control group (N=35) (Mean±SD)	P-value (Unpaired sample t test)
Age (Months) Weight (Kg) Height (cm) MAC	28.52±29.33 12.33±9.51 79.37±19.69 14.18±1.58	40.34±33.07 11.79±4.29 90.28±19.74 14.94±1.01	0.10 0.74 0.019 * 0.014 *
Sex Male N (%) Female N (%)	13 (32.5) 27 (67.5)	20 (57.89) 15 (42.10)	P value (Chi square test) P=0.029* P=0.014*

(P <0.05) is considered as statistically significant, *shows statistically significant P value)

Further subgroup analysis revealed that patients in study group had higher likelihood of having vitamin D deficiency as compared with controls. Similarly the level of vitamin D deficiency was also higher in study group (Table 2 and 3).

Table 2: Analysis of vitamin D deficiency amongst study and control groups.

Vitamin D level	Study group (N=40; %)	Control group (N= 35; %)	P-value (Z test for proportion)
Deficiency	37(92.5%)	16(45.71%)	Z=4.4** P<0.0001
Insufficiency	02(5%)	16(45.71%)	Z=4.1** P<0.0001
Sufficiency	01(2.5%)	03(8.57%)	P=0.24 (NS)

NS: Not significant *: significant ** highly significant

Table 3: Co-relation between vitamin d levels and distribution of ICU and IPD cases in study group.

Vitamin D level	No. of IPD admission	No. of ICU admission	Total
Deficiency	24(88%)	13(100%)	37
Insufficiency	02	0	02
Sufficiency	01	0	01
P value	<0.0001**	<0.0001**	40

By chi square test P value for number of IPD admissions in vitamin D deficient group is statistically significant (P<0.0001**) By chi square test P value for number of ICU admissions in vitamin D deficient group is statistically significant (P<0.0001), IPD: Indoor patient department ICU: intensive care unit

All patients with severe pneumonia needing ICU management were found to be deficient in vitamin D levels. None of the child with pneumonia admitted in ICU had normal or insufficient vitamin D levels (Table 3, 4). Regarding hemoglobin level, we noted that children with pneumonia were more likely to be anemic and were also more likely to have higher level of anemia.

Table 4: Contingency table-to see association of vitamin D level and occurrence of pneumonia cases.

	Present (n=40)	Absent (n=35)	P value by Fisher exact test
Deficiency	37	16	P<0.001**
Insufficiency	02	16	
Sufficiency	01	03	
Total	**40**	**35**	

P < 0.001*** (Highly significant)

Probability by Fisher Exact Test is P ≤0.001 considered highly significant. So that we can say that occurrence of Pneumonia is strongly associated with Vitamin D level. It can be seen from table above that occurrence is more in deficient group.

All children admitted to ICU had moderate to severe anemia (Table 5).

Table 5: Contingency table-to see association of Hb level and occurrence of pneumonia.

	Present (n=40)	Absent (n=40)	Total
Normal	01	05	06
Mild	04	06	10
Moderate	22	08	30
Severe	13	00	13
Total	40	19	59

P=0.003*** (Highly significant).

Probability by Fisher exact test is very low (P=0.003) considered statistically highly significant. So that we can say that there is significant association observed in Hb level and occurrence of Pneumonia. It can be seen from table that occurrence is more in severe group.

DISCUSSION

Before the discovery of antibiotics, sun exposure was very popular as a treatment of tuberculosis.[13] In fact, in 1903, Danish physician Niels Ryberg Finsen received the Nobel Prize for discovering light as a novel and effective treatment for skin tuberculosis.[14] It is a well-known fact that children with rickets are predisposed to develop pneumonia.[15] Haider N et al noted that 74% of children's with severe pneumonia had rickets as defined by low or normal calcium, low phosphorous and high serum alkaline phosphatase.[8] Even subclinical vitamin D deficiency was found to be associated with severe acute lower respiratory infection in Indian children less than 5

years of age.[16] The association of vitamin d deficiency and lower respiratory tract infections is not only limited to children but is also found amongst new borns, infants and adults.[17-19] Our study was aimed to detect prevalence of vitamin D deficiency in children admitted with pneumonia and also to study its impact on severity of pneumonia needing ICU admission. We excluded infants less than 6 months of age to avoid confounding effect of maternal vitamin D levels and maternal immunity of respiratory infections. Our study finding is with other authors that children with pneumonia had higher likelihood of having vitamin D deficiency as compared with controls.[6-9] Similarly, the level of vitamin D deficiency was also higher in study group.

The main criticism of above studies was possibility of acute illness altering the serum 25 (OH) D levels. To eliminate this confounding effect, Jovanovich AJ et al measured vitamin D levels at least 3 months prior to hospital admission.[7] They found that vitamin D levels < 37 ng/ml was associated with increased risk of community acquired pneumonia and level < 50 ng/ml was associated with sepsis. Thus borderline vitamin D deficiency may not predispose a child to develop pneumonia, but in a pneumonia child it may increase the severity of infection and sepsis. McNally et al observed no difference in vitamin D levels between the lower respiratory tract group and control group however they observed that significantly more children admitted to pediatric intensive care unit with acute lower respiratory tract infection had vitamin D deficiency.[20] These again confirm that children with vitamin D deficiency are more prone to develop severe infection needing ICU management. Inamo et al, who found that there was a significant correlation between vitamin D deficiency and the need for supplemental oxygen and ventilator management, also support this conclusion.[21]

Our study also revealed that subclinical vitamin D deficiency is more common in children with lower respiratory tract infection admitted in pediatric intensive care unit (p value <0.001). We noted that none of the children with pneumonia admitted in ICU had normal or insufficient vitamin D levels. All of them were found to be deficient in vitamin D. In contrast to our study, Tuğba Şişmanlar et al found no significant difference in vitamin D levels between the patient and control groups in their study.[22] It is important to note that all children included in this study were treated on outpatient basis. Authors also found high prevalence of vitamin D deficiency and insufficiency in both groups when compared to the previous studies conducted in their country and in the world. We presume that since all patients in this study were treated on outpatient basis, they had mild variety of pneumonia. This may reflect that vitamin D deficiency may not be more common in children with mild variety of pneumonia. In another study, no significant difference in vitamin D levels between cases and control group was observed when hospitalized cases of bronchiolitis and pneumonia were included in the study group.[23] But in this study, authors noted that Vitamin D deficiency and low hemoglobin level positively correlated with the severity of acute lower respiratory tract infections (ALRTIs). It might be interesting to explore whether Vitamin D deficiency has variable influence in predisposing a child to bacterial infections versus viral infections.[24]

Low hemoglobin level was found to be an independent risk factor for ALRTIs as it was detected in 56.25% of bronchiolitis cases, 62.5% of pneumonia cases and 42.71% of the control group (P = 0.044).[23] These data came in concordance with the study done by Harris et al.[25] These authors studied anemia and air pollution as risk factors for ARLTIs and found that anemic children were at increased risk of hospitalization after an acute respiratory infection when compared to healthy non-anemic children. They explained their findings by hypothesis that anemia independently decreases oxygen delivery to tissue thereby enhancing poor tissue oxygenation which remains a central pathophysiological deficit in ARTIs. In concordance with this study we noted that children with pneumonia were more likely to be anemic and were also more likely to have higher level of anemia. All children admitted to ICU in our study had Moderate to severe anemia.

Although Vitamin D replacement may not have any impact on severity or remission of existing acute lower respiratory tract infection, it may minimize the risk of recurrence in addition to providing other obvious benefit related to child's bone health. In a randomized control trial short-term supplementation with 2000 IU of vitamin D given orally for five days in children under 5 years suffering from severe pneumonia was not found to have any beneficial effect on resolution of severe pneumonia.[26] Another randomized study involving 453 children, of whom 224 children received single dose of 100,000 IU of oral vitamin D and 229 children received placebo failed to show any advantage of vitamin D therapy in terms of duration of recovery from severe pneumonia.[27] But it revealed lower recurrence rate in vitamin D supplemented group within 3 months of supplementation. It is likely that Vitamin D supplementation given during an acute episode of severe pneumonia played an immunomodulatory role, thereby increasing child's immune response to bacterial infection and thus minimizing risk of short-term recurrence. Discovery of vitamin D receptor (VDR) and 1 alpha hydroxylase in the cells of the immune system has made researchers to further explore role of vitamin D in modulating the immune response to various infectious diseases.[28] It is also known that vitamin D regulates the expression of specific endogenous antimicrobial peptides in immune cells.[3]

One important limitation of our study is that we did not evaluate impact of vitamin D supplementation on the future risk of recurrence. This is a topic of future research at our hospital. Inspite of this limitation we feel that our study adds to the existing evidence of association of

vitamin D deficiency and anemia in hospitalized children's below 5 years age suffering from pneumonia. Based on our study and existing medical literature, we feel that all the children presenting with pneumonia should have estimation of vitamin D levels done. Vitamin D supplementation is simple, safe and cheap method of correcting sub-clinical vitamin D deficiency in these children. Similarly, we strongly recommend correction of associated anemia in these children.

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