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A STUDY ON ASSOCIATION BETWEEN RESPIRATORY TRACT INFECTION AND SERUM 25(OH) VITAMIN D3 LEVEL IN CHILDREN OF A TERTIARY CARE HOSPITAL.

Biochemistry

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ABSTRACT

Respiratory tract infections (RTIs) are common worldwide and are responsible for significant morbidity and mortality. The most common causal agents are the bacterium Streptococcus pneumonia and influenza-virus. **Aims and Objectives:** To evaluate the level of serum 25(OH) Vitamin D3 in respiratory tract infection (both upper and lower) in children. **Materials and Methods:** A Case control study of total 105 cases of age group 2 months to 12 years from both indoor and OPD patients of Department of Pediatric Medicine, Patna Medical College and Hospital, Patna Bihar, were taken as cases who were suffering from respiratory tract infection and 86 disease free age and sex matched children were taken as control. **Results:** This study included total 196 children of age group 2 months to 12 years. 105 children who were suffering from respiratory tract infection were taken as cases and 86 disease free age and sex matched children were taken as controls. **Conclusion:** To conclude supplementation of vitamin D may be useful in prevention of RTIs, specially the LRTI group and to decrease the number of PICU admission.

KEYWORDS

Respiratory tract infection, Vitamin D deficiency.

INTRODUCTION:

Respiratory tract infections (RTIs) are common worldwide and are responsible for significant morbidity and mortality. The most common causal agents are the bacterium Streptococcus pneumonia and influenza-virus. The preventive measure by vaccination may not be completely protective due to non-responders and microbial vaccine escape mechanisms. Treatment options include symptomatic treatment, antibiotics and antiviral. Thus, additional ways to prevent or ameliorate RTIs are needed and modulation of the host immune response could provide such an innovative approach. Recent evidence suggests that vitamin D influences several immune pathways, with the net effect of boosting mucosal defence while simultaneously dampening excessive inflammation [1]. For example, vitamin D induces the gene encoding the antimicrobial peptide L3-L7 [2]. This peptide has potent bactericidal capacity against a number of important bacteria and viruses, including M. tuberculosis and influenza-virus [3,4].

In humans, the main source of vitamin D is UVB mediated synthesis in the skin. Certain food, such as oily fish and dairy products, contains vitamin D, but it is difficult to achieve sufficient intake by the diet alone. The activation of vitamin D involves two hydroxylation steps, one in the liver and one in the kidney. Vitamin D is transported bound to vitamin D binding protein to the liver, where 26 hydroxylase converts it into 25- hydroxyvitamin D, the most abundant circulating form of vitamin D [5]. Notably, the final activation of vitamin D, via 1-alpha hydroxylase (CYP27B1), also occurs in extra-renal tissues, including epithelial and immune cells [6]. In the respiratory tract, CYP27B1 is expressed in bronchial epithelial cells and induced by inflammatory stimuli [7].

Our humble intention in this study is to assess serum 25(OH) vitamin D_3 level in children as compared to age matched disease free control group of children and also to find out any correlation between serum 25-OH vitamin D_3 level with severity and frequency of respiratory tract infection in children.

Aims And Objectives:

1. To assess serum 25(OH) Vitamin D3 level in upper and lower respiratory tract infection in children.
2. To assess serum 25(OH) Vitamin D3 level in age sex matched control group and to compare it with the study group.
3. To assess correlation between serum 25(OH) Vitamin D3 level with severity and frequency of respiratory tract infections in children.

MATERIALS AND METHODS

A Hospital based Case Control study between age group 2 months to 12 years of both indoor and OPD patients of Department of Paediatric Medicine Patna Medical College and Hospital ,Patna ,Bihar, between February 2019 and July 2020 were taken as cases who were suffering from respiratory tract infection. Age and sex matched healthy children were taken as control.

INCLUSION CRITERIA:

All children between 2 months to 12 years of age attending the Department of Paediatric Medicine, Patna Medical College and Hospital, patna, having respiratory tract infection.

EXCLUSION CRITERIA-

1. Children having congenital heart disease.
2. Cases of childhood asthma and allergy.
3. Cases of tuberculosis
4. Children getting prophylactic vitamin D3 supplementation.
5. Children having immune-deficiency or getting immuno-suppressive therapy.

Parameters studied:

a) Clinical:

i. Detailed history ii. Detailed clinical examination

b) Laboratory Investigations:

i. Complete blood count
ii. Erythrocyte sedimentation rate (1 hour)
iii. Mantoux test
iv. X-ray - chest PA view
v. Serum 25(OH) Vitamin D3 level.

The children were classified into upper respiratory infection (URTI) and lower respiratory tract infections (LRTI) on the basis of clinical examination. A child with URTI of at least 6 times per year or LRTI of at least 2 times per year was defined as a patient with recurrent respiratory tract infection (RRI). The interval between every two infection was at least 7 days From the children of study group 2ml EDTA blood was taken for complete blood count and ESR. From the study and control group 3ml clotted blood were taken for estimation of serum 25(OH) vitamin D3 level. Mantoux test was done to exclude tuberculosis. Chest X-ray was done for diagnosis of lower respiratory tract infection.

Estimation Of 25(Oh) Vitamin D3:

Serum Vitamin D3 level of all the included patients and the normal control child between 2 months to 12 years of age were meticulously assessed . Three ml clotted blood sample was taken for measurement of serum Vitamin D3 (25OH) level from each of them.. Assessment of serum 25(OH) Vitamin D3 was done by the method of ELISA by EUROIMMUN kits.

Sample incubation:
200 µl of sample diluted in biotin/sample were pipetted, buffered into each of the microplate wells, incubated for 2 hours at room temperature(+18˚C to +25˚C). then the wells were subsequently washed 3 times using 300µl of working strength wash buffer for each wash.

Enzyme conjugate incubation:
100µl of enzyme conjugate (streptavidin-peroxidase) were pipetted into each of the microplate wells and incubated for 30 minutes at room temperature (+18˚C to +25˚C), and washed as earlier.

Substrate incubation:
100µl of chromogen/substrate solution pipetted into each of the microplate wells, incubated for 15 minutes at room temperature (+18˚C to +25˚C) (protected from direct sunlight).

Stopping the reaction:
100µl of stop solution were pipetted into each microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
photometric measurement of the colour intensity is made at wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Test is performed using fully automated analysis device.

Multiple methodologies for assessment of serum 25(OH) Vitamin D3 measurement exist, including RIA, HPLC, and liquid chromatography tandem mass spectroscopy [56,57]. The total 25(OH) vitamin D3 levels and 1,25(OH) vitamin D3 measured by high performance liquid chromatography (HPLC) or tandem mass spectrometry have been reported as the gold standard for vitamin D metabolite assay[55].

Data interpretation and Analysis:
For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS 20.0.1 and Graph Pad Prism version 5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables.

RESULTS:
This study included total 196 children of age group 2 months to 12 years. 105 children who were suffering from respiratory tract infection were taken as cases and 86 disease free age and sex matched children were taken as controls. The mean age of cases was 40.88±37.57 months and that of control was 37.66±35.25. Maximum population were in vitamin D insufficient state both in case and control. In cases, the number of vitamin D deficient and insufficient children was more as compared to control group. Among all age groups in cases, vitamin D was more deficient in infancy (40.5%) which is a positive finding signifying infants depend more on either sunlight exposure or dietary vitamin D to meet their requirement from birth. Maximum number of LRTI cases was below 60 months whereas URTI cases were above 60 months. This is one of the unique finding of my study.

Comparison of acute and recurrent RTI has no significant relation with serum vitamin D level, however inverse relation is found with recurrent state as compared to control. Recurrent RTI are more common in 12-60 month age group and vitamin D deficiency and insufficiency were more in this age group.

Table 1: Distribution of cases and control according to Age

	GROUP		
Age group	**Case**	**Control**	**TOTAL**
2 Months to 12 Months	35	30	65
Row %	53.8	46.2	100.0
Col %	33.3	34.9	34.0
12 Months to 60 Months	39	29	68
Row %	57.4	42.6	100.0
Col %	37.1	33.7	35.6
60 Months to 144 Months	31	27	58
Row %	53.4	46.6	100.0
Col %	29.5	31.4	30.4
TOTAL	105	86	191
Row %	55.0	45.0	100.0
Col %	100.0	100.0	100.0

Table 2: Distribution of vitamin D status in cases and control

	GROUP		
Vitamin D status	**Case(n=105)**	**Control (n=86)l**	**TOTAL**
Deficient(<20ng/ml)	42	4	46
Row %	91.3	8.7	100.0
Col %	40.0	4.7	24.1
Insufficient(21-29ng/ml)	58	58	116
Row %	50.0	50.0	100.0
Col %	55.2	67.4	60.7
Sufficient(>30ng/ml)	5	24	29
Row %	17.2	82.8	100.0
Col %	4.8	27.9	15.2
TOTAL	105	86	191
Row %	55.0	45.0	100.0
Col %	100.0	100.0	100.0

Chi-square value: 42.3688; p-value: <0.0001

Table 3: Distribution of cases according to type of RTI

Type of RTIs	No. of cases(n=105)	Percentage
LRTI	63	60.0%
URTI	42	40.0%
Total	105	100.0%

Out of 105 cases, 63(60%) were LRTI cases, 42(40%) were URTI.

Table 4: Distribution of mean vitamin D level in URTI and LRTI

		Number (n=105)	Mean (ng/ml)	SD (ng/ml)	Minimum (ng/ml)	Maximum (ng/ml)	Median (ng/ml)	p-value
LRTI	63	19.0167	5.7288	5.1000	34.0000	20.1000	0.0003	
URTI	42	22.9810	4.4712	14.2000	30.6000	22.2000		

DISCUSSION:
There are few studies that compared vitamin D with different types of respiratory tract infections from various western countries. Data from Indian studies are limited. We performed a case control study consisting of total 191 children of age group between 2 months to 144 months.

Total 105 cases having respiratory tract infection were chosen as cases while 86 healthy age and sex matched children were considered as controls. The mean age of our case group was 40.88±37.57 months in comparison to 37.66±35.25 in control group. In cases 58 (55.2%) patient belonged to male gender and 47 (44.8%) belonged to female gender, whereas in control group 33 (38.4%) belonged to female and 53 (61.6%) belonged to male. There was no significant difference in base line variables (age, gender) between the groups.

Out of 105 cases, majority belonged to age group 12-60 months (37.1%) followed by age group 2-12 months (33.3%) and rest were from 60 months to 144 months. Among the all age group in cases, vitamin D was more deficient in infant period (40.5%) which is a positive finding signifying infants depend on either sunlight exposure or dietary vitamin D to meet their requirement from birth.

Vitamin D is naturally deficient in human and cow's milk, thus it may predispose to vitamin D deficiency in younger children who are exclusively breast fed. Wayse V et al [10] in his study, found subclinical vitamin D deficiency and nonexclusive breastfeeding in the first 4 months is a significant risk factor for severe ALRI in Indian children. In our study the mean serum vitamin D level in exclusive breast fed child (19.90±6.33ng/ml) and in non breast fed child (20.90±5.25ng/ml) were mostly in insufficient and deficient reference range, hence no statistically significant difference of vitamin D level was noted in between these groups.(p=0.4015). Thus my study was in contrary to Wayse V et al [10] study.

In children, vitamin D deficiency and insufficiency are important health problems and variable prevalence's have been reported in studies conducted in many countries [10]. In a study conducted in Greeceby Lapatsanis D et al, vitamin D deficiency was found with a rate of 14% in children aged between three and 18 years [8]. In USA, in a study conducted byMansbach JM et al on serum 25-hydroxyvitamin D levels among US children aged 1 to 11 years, vitamin D deficiency was found with a rate of 14% and vitamin D insufficiency was found with a rate of 63% in 1799 children aged between one and five years [9].

Vitamin D plays a contributing role in immunity, it restores immune function and decreases cytokines level, vitamin D deficiency increases release of pro-inflammatory cytokines such as IL-6 and TNF-alpha In my study the mean serum vitamin D level was significantly decreased (p<0.0001) in cases (20.60±5.59) than that of control (26.38±4.58), which implies that vitamin D deficiency predisposes to respiratory tract infection in children.My study is almost similar to the findings in other studies by Velarde Lopez AA et al [5], Roth DE et al [5], Wayse V et al [4], Larkin A et al [1]. In all this studies they compared acute LRTI and mean vitamin D level and found a significant association of vitamin D deficiency with ALRI. However in none of the studies comparison of total RTIs including URTI and LRTI in case and control was done.

Ozdemir B et al [10] demonstrated vitamin D deficiency in children was associated with increased frequency of recurrent respiratory infections and chronic cough, with respect to healthy control. In my study the mean serum vitamin D level in acute case was 20.26± 5.45 and in recurrent was 21.41±5.91. However, when we tried to compare vitamin D levels between acute and recurrent RTI it did not show any significant difference in serum vitamin D levels (p=0.3393).

CONCLUSION:

Deficiency of serum level of 25(OH) Vitamin D3 is significantly associated with RTI, both URTI and LRTI. Deficiency of serum level of 25(OH) Vitamin D3 is more associated with LRTI as compared to URTI; however more studies are warranted to explore the association. No association was found with severity of pneumonia, Though there was significantly low level of serum 25(OH) Vitamin D3 was found in children admitted to PICU that may be due to other co-morbidities. To conclude supplementation of vitamin D may be useful in prevention of RTIs, specially the LRTI group and to decrease the number of PICU admission. However more studies are needed to be performed in this field to assess further correlation with frequency, severity and the types of RTIs.

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