



## Relationship between Vitamin D Levels and Outcome of Pneumonia in Children

*Relation entre le taux sanguin de la vitamine D et la survenue de pneumonie chez les enfants*

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### ABSTRACT

**BACKGROUND:** Pneumonia, a common childhood infection in Nigerian children with a number of debilitating complications such as empyema thoracis, has been linked to vitamin D deficiency due to its ability to modulate the T lymphocyte of the immune system .

**OBJECTIVE:** To determine the relationship between vitamin D and outcome of pneumonia in children.

**METHODS:** This was a case-control study involving 24 children, admitted for pneumonia as subjects and 10 children without pneumonia as controls. Pre-formatted questionnaire was utilized to obtain background information, anthropometric measurements were made to determine nutritional status and estimation of 25-hydroxy cholecalciferol (25OHD) done for all those studied.

**RESULTS:** The mean (SD) serum 25OHD concentration was 104 (59) nmol/L and 130 (107) nmol/L for subjects and controls respectively. Amongst the subjects 15 (54%) had serum 25OHD less than 70nmol/L and 11 (46%) serum 25OHD greater than 70nmol/L. Hypocalcaemia was present in 15 (54%) of the subjects. Further analysis of hypocalcaemia with regards to the concentration of serum 25OHD showed that 2 (13%) had levels below 40nmol/L, 10 (67%) had levels below 70nmol/L and 3 (20%) above 70nmol/L. Hypocalcaemia was more frequent among subjects with 25OHD below 70nmol/L compared with those above 70nmol/L, ( $p = 0.01$ ). Empyema thoracis and death occurred amongst the two subjects with 25OHD between 27.5 and 40nmol/L. Anaemia was more frequent among subjects with 25OHD below 70nmol/L compared with those above 70nmol/L ( $p = 0.03$ ).

**CONCLUSION:** The study showed that Vitamin D insufficiency, and not solely its deficiency, may have an important role to play in the immune and haemopoietic system. It may therefore affect the response of a child to infections especially pneumonia. *WAJM 2010; 29(6): 373–378.*

Keywords: Vitamin D, Children, Pneumonia.

### RÉSUMÉ

**CONTEXTE:** La pneumonie, une infection commune chez les enfants africains avec un certain nombre de complications telles que l'empyème thoracique, est associée à l'insuffisance de la vitamine D. La vitamine D intervient dans la modulation du système immunitaire, en particulier du lymphocyte T.

**OBJECTIFS:** déterminer le rapport entre le taux de la vitamine D dans le sang et le nombre de cas de pneumonie chez les enfants.

**MÉTHODES:** il s'agissait d'une étude cas-témoins intéressant 24 enfants admis pour une pneumonie et 10 enfants-témoins, sans pneumonie. Un questionnaire préétabli a été utilisé pour le recueil des données. Des mesures anthropométriques ont été effectuées pour déterminer le statut nutritionnel et le taux d'hydroxycholecalciferol (25OHD) dans le sérum chez tous les enfants.

**RÉSULTATS:** La concentration sanguine moyenne de 25OHD était 104 (59) nmol/L et 130 (107) nmol/L respectivement chez les patients et les témoins. Parmi les patients, 15 (54%) ont eu un taux de 25OHD inférieur à 70nmol/L, et 11 (de 46%) un taux supérieur à 70 nmol/L. L'hypocalcémie était présente chez 15 (54%) patients. Parmi les patients qui avaient une hypocalcémie, 2(13%) avaient des taux de 25OHD en dessous de 40nmol/L, 10 (67%) en dessous de 70nmol/L et 3 (20%) au dessus de 70 nmol/L. L'hypocalcémie était plus fréquente chez les patients avec un taux de 25OHD en dessous de 70nmol/L, comparé à ceux qui avaient un taux supérieur à 70 nmol/L ( $p = 0,01$ ). L'empyème thoracique et la mort se sont produits chez les 2 patients qui avait un taux de 25OHD entre 27,5 et 40 nmol/L. L'anémie était plus fréquente chez les patients qui avaient un taux de 25OHD en dessous de 70nmol/L comparé à ceux qui avaient un taux supérieur à 70 nmol/L ( $p = 0,03$ ).

**CONCLUSION:** Les résultats de cette étude montrent qu'une insuffisance en vitamine D, plus qu'un simple déficit, joue un rôle important dans les systèmes immunitaire et hématopoïétique. Elle peut donc affecter la réponse des enfants aux infections particulièrement la pneumonie. *WAJM 2010; 29 (6): 373–378.*

Mots-clés: Vitamine D, enfants, pneumonie.

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Abbreviations: 25OHD, Hydroxy cholecalciferol; CI, Confidence interval; LASUTH, Lagos State University Teaching Hospital; LUTH, Lagos University Teaching Hospital.

## INTRODUCTION

The United Nations Children Fund (UNICEF) 2008 report states that 32% of Under-5 Nigerian children were admitted for pneumonia, thus making it an important cause of mortality and morbidity in these children.<sup>1</sup> The following factors are known to increase the risk for pneumonia, its complication and mortality. These include environmental factors such as indoor air pollutants from cooking inside the house with wood or dung, or tobacco smoking by parents; increased transmission of pathogens due to overcrowding and poor ventilation at home; childcare practices like reduced child spacing early introduction of child in an overcrowded, poor facility daycare and finally malnutrition from varied causes such as deficiency of calorie, protein, micronutrient and vitamins.<sup>2,3</sup>

The common causes of nutritional rickets in Nigeria are calcium and vitamin D deficiency.<sup>4-7</sup> They are without difficulty recognized clinically and are inexpensive to manage. Intake of adequate and good quality food with appropriate period of exposure time to sun will rectify these deficiency. For diagnosis of vitamin D deficiency in children and adolescents, the widely used cut-off value is serum 25(OH) D levels of 27.50nmol/L or less.<sup>8</sup> For children and adolescent the cut off point value used for vitamin D insufficiency is that of the adult until a consensus is reached, The value for adults is currently 70 nmol/L or less,<sup>9,10</sup> this higher than what was used in earlier literature where Vitamin D insufficiency was defined as 50nmol or less.<sup>8,11-13</sup> Vitamin D insufficiency, has been shown to have a deleterious effect on skeletal health of adults<sup>11-14</sup> and probably too in children and adolescents by stunting of growth.<sup>15,16</sup> Unfortunately it can be diagnosed only laboratory means t now since there isn't any known typical characteristic clinical features

This case control study was carried out to test the hypotheses that vitamin D deficiency or insufficiency predisposes a child to pneumonia and that very low serum vitamin D level increases the prevalence of complications arising from pneumonia.

## SUBJECTS, MATERIALS, AND METHODS

The study was a cross-sectional case control study conducted between January and mid February (6 weeks) during the dry dusty season with little rainfall in Nigeria. Three hospitals (Lagos University Teaching Hospital (LUTH), Lagos State University Teaching Hospital (LASUTH) and Massey Children Hospital Lagos) were used for recruitment of subjects. The three hospitals chosen had high admission rates and were situated in three different densely populated areas of Lagos metropolis. Lagos is a port and the second most populous conurbation in Nigeria. It is currently the second most populous city in Africa. It is a huge metropolis which originated on islands separated by creeks, such as Lagos Island, that fringe the southwest mouth of Lagos Lagoon, protected from the Atlantic Ocean by long sand spits such as Bar Beach which stretch up to 100 km east and west of the mouth. The city is the economic and financial capital of Nigeria.<sup>17</sup>

### Subject Enrollment

Children between the ages of two months and five years who were admitted into the wards of the three hospitals within six weeks from January to the middle of February with either lobar or bronchial pneumonia were enrolled into the study. The child to be enrolled into the study must fulfilled the following inclusion criteria.

### Inclusion Criteria

The child must have the following clinical features before the diagnosis of pneumonia was made. There must be tachypnoea (respiratory rate >50 cycle/minute for children 2-11 months or >40 cycles/minutes for children aged 1-5 years). There must be chest in-drawing with crackles on auscultation and/or radiographic findings of alveolar infiltrates or air bronchogram.<sup>18</sup>

Apart from above the subject included must be aged between two months to 60 months and be on admission on the ward. Also subject must be treated with the same antibiotics (ampicillin and cloxacillin mixture) and be

given the appropriate dosage for weight. Parents must give consent for inclusion of ward into the study. Child must have a negative radiological survey and evidence of BCG immunization scar to eliminate tuberculosis infection.

### Exclusion Criteria

Any subject who had the following was excluded. Vitamin D and alkaline phosphatase are two important biochemical parameters in the study both have part of their metabolism occurring in the liver. Therefore any child with a history of jaundice was excluded. Also any child with history of asthma, croup, acute thoracic problems apart from pneumonia, chronic diarrhea, congenital heart disease or drugs that could be associated with rickets like phenobarbitone were excluded.

### Control Subjects Enrollment

Controls were children admitted during the same period without pneumonia or any of the exclusion criteria.

### Methods

The following laboratory parameters were measured in the Department of Pediatric research laboratory at Lagos University Teaching Hospital using the laboratory standardized methods and quality assurance: Serum alkaline phosphatase, calcium, and phosphate. The haematological indices were done using standard methods.

The vitamin D estimations were done at the chemical pathology laboratory of Mount Sinai hospital Toronto Canada in collaboration with Vieth R, using the radioimmunoassay (Dia-Sorin, Stillwater, MN).

A pre-formatted questionnaire to obtain background information about diet, medications, breast-feeding, socioeconomic status, length of exposure to sunlight daily, history of bone fracture either spontaneous or following trauma and frequency of symptoms of acute respiratory infection such as cough, running nose, sore throat, fast breathing, chest pain with or without an associated fever was completed by the attending physician. A diagnosis of pneumonia was made using the inclusion criteria strictly.

The anthropometric measurements including weight and height (or length depending on subjects' age) were performed using standard measuring appliances and methods by one trained identified staff at recruiting hospital. Anthropometric measurement collected were placed on the CDC <sup>19</sup> growth chart to determine the subjects' nutritional status.

Informed consent was obtained from the caretaker before any child was included, blood withdrawn for biochemical analysis or application of questionnaire applied. All children diagnosed with pneumonia were seen and treated with appropriate management without bias to inclusion or otherwise.

The study was approved by the ethical committees of the Lagos University Teaching Hospital (LUTH) and Lagos State University Teaching Hospital (LASUTH).

Statistical analysis was carried out using the student's t-test and Fishers exact test. Confidence interval was set at 95% and level of significance was set at p less or equal to 0.05.using the microsoft excel 2007 software

## RESULTS

There were 522 children admitted altogether into the three hospitals during the period of study and 463 (87%) were between ages two to 60 months. Clinical features of pneumonia were diagnosed in 32 (7%) using the inclusion criteria, but only 24 (75%) were eligible for analysis. Five of the subjects were excluded for lysed blood samples. Consent was rescinded for two after initial agreement and were thus excluded. Another was excluded for having haemoglobinopathy SS.

Ten children whose parents consented and fulfilled both the inclusion and exclusion criteria were enrolled. They were aged between 2 months to 60 months. Eight were admitted for malaria fever and two had meningitis.

### Distribution of Subjects per Participating Health Facility

Lagos University Teaching Hospital accounted for 11 of the 32 subjects diagnosed with pneumonia while eight

came from the Massey Children Hospital and 13 from Lagos State University Teaching Hospital.

### Subject and Control Characteristics

Table 1 shows the basic characteristics of the subjects and controls. There was no significant difference between the subjects and controls in weight or height. The lengths of time of exposure to sunlight in both groups which were three and four hours in subjects and controls, respectively, were significantly different. The mean period for exclusive breast feeding was the same in both groups (three months).

Sixty percent of subjects and controls between ages six and 12 months were on complementary meals to which formula milk was added four to six times a day. The subjects who were older than 12 months of age usually had 2–3 tablespoonfuls of milk added to their pap or tea per day. Irrespective of age, 14(60%) and 8(80%) of subjects and controls respectively were on various types of multivitamins with vitamin D

concentration in the preparation varying between 50IU to 200IU per five mls. Mothers gave on the average 5mls of preparation once a day, but none was on calcium supplement. Some mothers [20 (60%)] had sample of vitamins supplement with them for verification.

### Serum Vitamin D Profile

The subjects' mean serum 25 hydroxyvitamin D (25OHD) was 104 (59) nmol/L while that of control was 130 (107) nmol/L, p=.0, 50 not significant. Table 2 shows that none of the subjects had serum 25OHD less than 27.5nmol/L, but two (8%) had 25OHD less than 40nmol/L (these two were not on multivitamin tonics). In total 13(54%) subjects had serum 25OHD less than 70nmol/L, while 11 (46%) had serum 25OHD levels greater than 70nmol/L (Table 3). Amongst the control group, three (30%) had 25OHD less than 70nmol/L of which one had 25OHD less than 27.5nmol/L and six (60%) had 25OHD greater than 70nmol/L (Table 3).

**Table 1: Characteristics of Children with Pneumonia and Controls**

Characteristic	Children with Pneumonia	Control Children	P value
Age months, mean (range)	20 (6–30)	21 (6–48)	NS
Sex M:F	19:15	5:5	
Height (cm)	77 (12.4)	91 (32.5)	NS
Weight (kg)	9.0 (3.0)	11.0 (3.0)	NS
Weekly exposure (hours)	3 (0.9)	4 (1.2)	NS

Values as mean (SD) except age and sex.

**Table 2: Vitamin D Concentrations in Children by Pneumonia Status**

25OHD Concentration (nmol/L)	Children with* Pneumonia (n=24)	Control* Children (n=10)	Confidence Interval (95% CI)	P value
<27.5	0 (0)	1 (10)	–0.1491–0.3774	0.30
27.5–40	2 (8)	0 (0)	–0.2006–0.2358	0.50
27.5–70	13 (54)	3 (30)	–0.1167–0.5046	0.4
>70	11 (46)	6 (60)	–0.2033–0.4348	0.7

\* Reported as n (%)

**Table 3: Biochemical Characteristics of Study Groups \***

Plasma Analyte	Children with Pneumonia (n=24)	Control Children (n=10)	Mean Difference (95% CI)	P value
25OHD nmol/L	104 (59)	130 (107)	26.4 (-57.8-110.5)	0.50
Corrected Calcium (mmol/L)	2.04 (0.36)	1.94 (0.22)	-0.11 (-0.38-0.17)	0.43
Phosphate (mmol/L)	7.7 (1.55)	2.1 (1.6)	-5.53 (-13.3-2.2)	0.15
Alkaline Phosphatase (U/L)	220 (138)	160 (148)	-60 (-2.14-94)	0.43
Magnesium (mmol/L)	0.95 (0.20)	0.74 (0.14)	-0.21 (-0.35-0.06)	0.06
Total protein (g/L)	64 (25)	65 (12)	0.7 (-14-15)	0.92
Albumin (g/L)	35 (6.6)	34 (7.7)	-1.8 (-8.3-4.6)	0.55

\* Mean (SD). Equal variances are not assumed for t-test of significance

**Table 4: Complications in Children with Pneumonia by Values of Vitamin D\***

Complication	25OHD (< 40)	25OHD (40<70)	25OHDX (40<70)	95% CI	Fishers Exact Test (p)
N	2	13	11		
Empyema					
Thoracis	2 (100)	3 (23)	0 (0)	-0.0679-0.5026	0.7
Anemia	2 (100)	11 (79)	4 (36)	0.0135-0.0647	0.03
Death	1 (50)	2 (15)	0(0)	-0.127 -0.4223	0.48
Hypocalcemia	2 (100)	10 (79)	3 (27)	0.0412-0.758	0.01

\* reported as n (%); vitamin levels as nmol/L.

### Serum Vitamin D Profile and Calcium

The mean serum calcium concentration did not differ significantly between the two groups,  $p=0.43$  (Table 3). Amongst the subjects with pneumonia, 13 (54%) had hypocalcaemia. From the thirteen of the subjects with hypocalcaemia<sup>8</sup> 77% subjects had serum 25OHD less than 70nmol/L. It was interesting to note that both subjects with serum 25OHD less than 40nmol/L had hypocalcaemia. The remaining three (23%) subjects with hypocalcaemia were those with serum 25OHD greater than 70nmol/L. Comparing the proportion of subjects with 25OHD less than 70nmol/L with those with 25OHD greater than 70nmol/L showed statistical significance ( $p=0.01$ ).

### Serum Vitamin D Profile and Complications

The serum 25OHD of the two patients with empyema thoracis was less than 40nmol/L, so also was the patient

with pneumonia who died. Anaemia (haemoglobin <10gs/dl) was found in 15 (62.5%) children with pneumonia. Eleven (84%) of these 15 children had serum 25OHD less than 70nmol/L. It was also noted that the two children with serum 25OHD less than 40nmol/L as has been noted for other complication also were anaemic, while only four were anaemic amongst those who had 25OHD greater than 70nmol/L. Comparing the proportion of subjects with serum 25OHD less than 70nmol/L and those with serum 25OHD greater than 70nmol/L was statistically significant difference at 95% CI ( $p=0.03$ ) (Table 4).

### DISCUSSION

Vitamin D deficiency is common in many parts of the world<sup>2-5, 19-23</sup> and resurgence is being observed in places where it had been previously rare.<sup>20</sup> Vitamin D deficiency is diagnosed when the serum 25OHD is less than 27.5nmol/L, and presents vividly clinically as rickets

or osteomalacia depending on age. However, vitamin D insufficiency, that is 25OHD less than 70nmol/L, has clinical presentation that is obscure and its effect on health is usually not appreciated.<sup>24</sup>

Although the effect of vitamin D insufficiency has been appreciated in adults<sup>10, 11, 13, 15</sup> its not so in children.<sup>14</sup> Vitamin D nutrition has been discovered to affect health beyond just bone.<sup>6,16,24-26</sup> It does this through signaling mechanisms mediated locally by circulating 25OHD. Many tissues have 1, 25 dihydroxyvitamin-1 alpha hydroxylase (1,25(OH)<sub>2</sub>D-1 $\alpha$ OH) enzyme or receptors for 1,25(OH)<sub>2</sub>D.<sup>26,27</sup> These include the skin, pancreas (islets), brain, adrenal medulla, prostate, breast and colon. Through many studies correlation has been found between vitamin D deficiency and pneumonia, tuberculosis, diabetes, cancer (prostate and breast), multiple sclerosis and hypertension.<sup>6,24-30</sup>

In the haemopoietic cells of the bone marrow, 1,25(OH)<sub>2</sub>D promotes the differentiation of promonocytes into monocytes, megalocytes and finally into osteoclasts. It has been shown that interferon, produced by activated T-lymphocytes mediates production of 1,25(OH)<sub>2</sub>D-1 $\alpha$ OH, conferring on these cells the ability to produce limited 1,25(OH)<sub>2</sub>D. This indicates an involvement of 1,25(OH)<sub>2</sub>D in cell differentiation in the bone marrow, the immune system and the epidermis. Though still being investigated, mild vitamin D deficiency may have great impact on mortality and morbidity of a growing child,<sup>14-16</sup> especially in the developing world where nutritional deficiency still has a big role to play. Vitamin D deficiency or insufficiency is one of the nutritional deficiencies that can easily be corrected cheaply. Consumption of the daily requirement or exposure of adequate skin surface area at the appropriate time for at least 15-30 minutes per day should prevent vitamin D deficiency but not necessarily insufficiency.

Studies done in Nigeria have shown that calcium deficiency and not vitamin D deficiency is the common cause of nutritional rickets.<sup>2-5,31</sup> Vitamin D supplementation cannot therefore be justified as a mandatory addition to our national health policy as it is being done in

Europe, Canada and the American countries.<sup>32</sup> It can be justifiably assumed that since we are living in the tropical zone of the world, adequate exposure to sunlight by the children should be sufficient to prevent vitamin D deficiency. This assumption is supported even by the present study as vitamin D deficiency was seen in only 20% of the patients studied. It is pertinent to take cognizance of the claim by almost 75% of the parents of subjects of administering multivitamin at home (although only 60% could be verified) and the fact that, those with vitamin D deficiency were those who were not on multivitamin. It should be noted that, although the children had adequate exposure time to sunlight, reduced skin area exposed because of clothing and the timing of exposure may probably explain the high prevalence of vitamin D insufficiency amongst even the controls (33%). Although there were no statistically significant differences in complications of pneumonia such as empyema thoracis and death, the fact that this was observed in those whose 25OHD level were less than 40nmol/L shows that vitamin D may have a role to play through the immune system. Also, hypocalcaemia and anaemia prevalence was high amongst the children with pneumonia and with serum 25OHD less than 70nmol/l (that is vitamin D insufficiency). Comparison these parameters in those with vitamin D insufficiency with those who were vitamin D sufficient was statistically significant.

The observations made are consistent with our hypotheses that vitamin D insufficiency and not just deficiency may have an important role to play in the immune and haemopoietic system and therefore affect the response of a child to infection.

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