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Vitamin D insufficiency among hospitalised children in the Northern Territory

Original article

Michael J Binks[1], Heidi C Smith-Vaughan[1], Naor Bar-Zeev[1], Anne B Chang[1,2], Ross M Andrews[1]

1. Menzies School of Health Research, Charles Darwin University, Darwin, Australia

2. Queensland Children's Respiratory Centre, Queensland Medical Research Institute, Royal Children's Hospital, Brisbane, Queensland, Australia

[§]**Corresponding Author.**

Michael Binks

PO BOX 41096

Phone +61 8 8922 6965

Fax +61 8 8927 5187

Email: michael.binks@menzies.edu.au

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Abstract

Introduction:

Acute lower respiratory infections (ALRI's) are the most common reason for hospitalisation of young children in the Northern Territory of Australia. International studies have linked vitamin D deficiency with increased risk of ALRI in paediatric populations but this has not been explored in tropical regions such as the Top End of the Northern Territory.

Aim:

To determine the prevalence of vitamin D insufficiency amongst children hospitalised with ALRI in the Northern Territory.

Methods:

Vitamin D serum metabolite (25OHD3) levels were retrospectively measured using liquid chromatography–mass spectrometry in 74 children (64% Male; 57% Indigenous) aged less than 3 years admitted to Royal Darwin Hospital in the Northern Territory of Australia between May 2008 and May 2010.

Results:

There were 44 (59%) ALRI classified hospitalisations and 30 (41%) non-ALRI classified hospitalisations. The most common ALRI diagnoses were bronchiolitis (n=22, 30%) and pneumonia (n=21, 28%) whilst the most common non-ALRI diagnosis was gastroenteritis (n=20, 27%). Overall, 24/74 (32%) children had 25OHD3 levels <75nmol/L (insufficiency). For children hospitalised with ALRI, 23% (10/44) had

vitamin D insufficiency compared with 47% (14/30) among children hospitalised for other reasons (OR 0.34, 95%CI 0.11 to 1.03; p=0.043). Twelve of the 20 (60%) children hospitalised for gastroenteritis had vitamin D insufficiency.

Conclusions:

Vitamin D insufficiency was observed in almost one third of these hospitalised children. Children hospitalised with an ALRI were less likely to have vitamin D insufficiency compared to children hospitalised for other conditions (predominantly gastroenteritis).

Introduction

Respiratory diseases are a significant health problem in the Northern Territory representing the largest cause of preventable mortality in infants[1]. Between 1999 and 2004, over 22% of Indigenous infants were hospitalised at least once with an acute lower respiratory infection (ALRI) before 12 months of age[2]. High rates of bronchiectasis among this population are mostly related to recurrent ALRI[3].

Several studies have demonstrated an inverse association between vitamin D levels and ALRI in children even in regions with abundant sunshine[4,5]. In Bangladesh where infants (1-18 months) have serum vitamin D metabolite (25OHD3) levels <50nmol/L despite abundant year round sunshine[4], conditional logistic regression showed the odds of ALRI hospitalisation among children halved for each 10nmol/L increase in 25OHD3. In the cooler, seasonal location of New Zealand, cord blood 25OHD3 levels below 75nmol/L were associated with a higher risk of respiratory infection at 3 months of age[6]. More recently, a randomised controlled trial (RCT) showed that daily ingestion of vitamin D fortified milk (300IU) reduced the risk of acute respiratory infections in Mongolian children with deficient baseline 25OHD3 levels[7]. Not all studies support a role for vitamin D in preventing respiratory infection. A large RCT (n=3046) showed no benefit of 3 monthly bolus doses of vitamin D (100 000IU) against pneumonia incidence among children (1-11 months of age) in inner-city districts of Kabul where both pneumonia and vitamin D deficiency are common[8].

There is ongoing controversy about which vitamin D cut-offs should be used. In the United States serum 25OHD3 levels below 50nmol/L are considered deficient, 50-74nmol/L as insufficient and ≥75nmol/L as optimal[9], whereas in Australia and the

United Kingdom levels above 50nmol/L are generally considered adequate[10,11]. The evidence does suggest however, that an increased risk of respiratory infection exists for 25OHD3 levels up to 75nmol/L[6,12,13]. Known risk factors for vitamin D deficiency include obesity, premature birth, pigmented skin, low sun exposure or southerly latitude, and malabsorption[10]. Breast fed infants of vitamin D deficient mothers may also be at increased risk of deficiency or insufficiency[14].

Despite the respiratory disease burden among Australian children in the Northern Territory, there are no published data relating vitamin D status to ALRI in this setting. We describe 25OHD3 levels among 74 children (<3 years old) who were hospitalised in the Northern Territory with an episode of ALRI or for other conditions.

Methods

Design and Setting

Cross sectional study of a convenience sample of children aged <3 years who were admitted to Royal Darwin Hospital, Northern Territory of Australia, from May 2008 - May 2010.

Seventy four children hospitalised with respiratory or other illnesses had sufficient stored blood collected (≥100µl at -80^{0}C) for vitamin D testing. Testing was performed

following approval by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC 07/33).

Hospitalisation diagnosis

We investigated all diagnostic ICD-10-AM codes[15] recorded in the Northern Territory hospital discharge dataset at Royal Darwin Hospital for the episode of hospitalisation corresponding with each serum sample. All 74 samples were from first hospitalisation episodes. ICD-10-AM codes J09-J18.9, J20-J22 or A37.0 were used to define ALRI[15].

Serum vitamin D measurements

Serum 25OHD3 levels below 75nmol/L were used to define vitamin D insufficiency[9]. Vitamin D assays were performed by Royal Melbourne Institute of Technology Drug Discovery Technologies Pty Ltd (Melbourne, Australia) in January 2011. Levels of 25OHD3 were measured in 100µl of serum using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) as previously published[16]. Assay precision was determined using low, medium and high commercial controls (UTAK, Australia) and sample identification was concealed during testing.

Statistical analysis

For the primary analysis we compared the proportions of vitamin D insufficiency in children hospitalised for an ALRI (versus no ALRI) and also for children hospitalised with gastroenteritis (versus no gastroenteritis). Odds ratios are reported, Fisher's exact test p-values <0.05 were considered statistically significant. Comparisons of serum 25OHD3 levels were assessed using the Student's T-test. Weight (at hospital discharge) for age z-scores (standard deviations) were calculated with reference to World Health Organization (WHO) child growth standards using the STATA module "zscore06"[17]. A weight for age z-score >2 standard deviations below the WHO reference median[18] was considered as severely underweight and indicative of under-nutrition. Haemoglobin values were considered low if <110 g/L for all ages[19]. All statistical analyses were performed using STATA 12 (Stata Corp, USA).

Results

Hospitalisation diagnosis

Among the 74 hospitalised study children there were 124 ICD-10-AM discharge diagnoses (average 1.7 diagnoses per child). Two participants coded solely with ICD-10-AM codes R05 (cough) and J98.4 (other lung disorders) were classified as ALRI following a clinical note review. The most common reasons for hospitalisation were: ALRI (59%), gastroenteritis (27%), upper respiratory tract infection (19%) and anaemia (18%). The most common ALRI diagnoses were bronchiolitis (30%) and pneumonia (28%) (Table 1).

Characteristics of study participants

The median age at hospitalisation was 6 months (range 1, 31), 47 (64%) were male, 42 (57%) were Indigenous and the median hospital stay was 5 days (range 1, 32). Those children hospitalised with ALRI were more likely to attend childcare (25% versus 3%, p=0.020) than those hospitalised for other reasons. Whereas those children hospitalised with gastroenteritis were more likely to be Indigenous (85% versus 46%, p=0.003) and more likely to live in a remote location (80% versus 43%, p=0.008) when compared with those children hospitalised for reasons excluding gastroenteritis. No other factors were identified as being associated with ALRI compared to non-ALRI admissions or with gastroenteritis compared to non-gastroenteritis admissions.

Accuracy and validity of the vitamin D assay

The ID-LC-MS/MS vitamin D assay fulfilled the quality control acceptance criteria. The coefficient of variance for the triplicate determinations of low, medium and high commercial controls was 6.4%, 6.9% and 3.9% respectively and the mean for each quality control (QC) level was within 3 standard deviations of the historical data for the corresponding QC lot number. The calibration curve had a regression coefficient (R^2) value of 0.996.

Vitamin D levels and participant characteristics

Mean 25OHD3 for all 74 children was 83.9nmol/L; 24/74 (32%) children were vitamin D insufficient (<75nmol/L)and 11/74 (15%) were deficient (<50nmol/L). Mean serum 25OHD3 levels (Table 2) were significantly lower for infants who were breast feeding when hospitalised (78 versus 93nmol/L; p=0.029). There was no difference in mean serum 25OHD3 levels for Indigenous infants who were breast feeding compared to those Indigenous infants who were not, but among the 32 non-Indigenous infants the mean 25OHD3 levels were 102nmol/L for non-breast feeding children compared with 78nmol/L for breast feeding children (both mean levels being above the insufficiency cut-off). Mean 25OHD3 levels during hospitalisation were significantly lower for infants with a history of pre-term birth (70 versus 91nmol/L; p=0.013) compared to those who were full term (not pre-term). The mean 25OHD3 levels for Indigenous compared to non-Indigenous children (78 versus 91nmol/L; p=0.063) and for children living in remote (81% Indigenous) compared to urban settings (78 versus 90nmol/L; p=0.074) were lower but not statistically different. We found no other correlation between the demographics and 25OHD3 levels.

Comparison of vitamin D insufficiency by hospitalisation diagnosis

Among 44 ALRI hospitalised children, 10 (23%) had serum 25OHD3 levels below 75nmol/L compared to 14/30 (47%) non-ALRI children (p=0.043) (Table 3). Among 20 gastroenteritis hospitalised children, 12 (60%) had 25OHD3 levels below 75nmol/L compared to 12/54 (22%) non-gastroenteritis children (p=0.004). Six children had a co-diagnosis of ALRI and gastroenteritis, of whom four had 25OHD3 levels below

75nmol/L. Neither the omission of these co-diagnosed children nor the use of a 50nmol/L cutoff had a meaningful influence on the outcomes.

Within the ALRI group there were 22 (16 exclusive) bronchiolitis diagnoses and 21 (14 exclusive) pneumonia diagnoses. In comparison to the 14/30 (47%) non-ALRI children with insufficient serum 25OHD3 levels (<75nmol/L), there was a similar proportion of vitamin D insufficiency (<75nmol/L) among children with an exclusive diagnosis of bronchiolitis (7/16 (44%); p=1.0) but a lower proportion of vitamin D insufficiency (<75nmol/L) among children with an exclusive diagnosis of pneumonia (2/14 (14%); p=0.049). Care needs to be taken in interpretation of the subgroup analyses due to small numbers and borderline significance.

Vitamin D levels and hospitalisation diagnosis

Mean serum 25OHD3 levels were significantly higher in the ALRI compared to the non-ALRI diagnosed children (90 versus 75nmol/L respectively; p=0.020) with variation evident among the sub-diagnoses, bronchiolitis (76nmol/L) and pneumonia (94nmol/L) (Figure 1). In contrast, the mean serum 25OHD3 were significantly lower in the gastroenteritis compared to non-gastroenteritis children (68 versus 90nmol/L respectively; p=0.003). For the six children where ALRI and gastroenteritis occurred together the median 25OHD3 level was 59nmol/L.

Discussion

Our study is the first to examine the association between ALRI and vitamin D insufficiency in children of the Northern Territory. In this small hospital-based study, we found that almost one-third (32%) of children hospitalised for predominantly infectious causes had suboptimal 25OHD3 levels (<75nmol/L).

The Northern Territory has some of the highest rates of ALRI hospitalisation in the world, especially for young Indigenous children (22%)[2]. Together with studies reporting an association between vitamin D insufficiency, mucosal immunity and the risk of ALRI[4,20], and the absence of vitamin D data in the Northern Territory, we investigated vitamin D insufficiency among a convenience sample of hospitalised children for whom the most common diagnoses were ALRI and gastroenteritis. We found a high proportion of vitamin D insufficiency among children hospitalised with ALRI (23%) and an even higher proportion among those hospitalised for gastroenteritis (60%).

The higher point prevalence of vitamin D insufficiency among the non-ALRI diagnosed children was likely influenced by lower 25OHD3 levels in the non-ALRI children with gastroenteritis compared to without gastroenteritis (65 versus 83nmol/L respectively; p=0.106). Among all 74 children the 25OHD3 levels were significantly lower in gastroenteritis compared to non-gastroenteritis hospitalisations (68 versus 90nmol/L respectively; p=0.003), noting that remote living and Indigeneity were more common among gastroenteritis diagnosed children and may be confounders of this association. Whilst vitamin D insufficiency could be considered a risk factor for gastroenteritis because most mucosal surfaces rely, at least partly, on vitamin D-mediated immune defence[21], poor dietary absorption and a greater burden of illness among these children may also explain the lower vitamin D levels.

In the northernmost regions of the Northern Territory, sunshine hours and UV index are well above the national average, however, population data on vitamin D in this region are lacking. In a study from far North Queensland, only 7% of 116 women presenting for antenatal care were vitamin D insufficient (<75nmol/L), though Indigenous women had significantly lower 25OHD3 levels, with the median just above 75nmol/L[22]. In 2011, a national survey of Australian adults over 25 years of age (n=11,247) found 73% insufficiency (<75nmol/L) overall[23]. A subset of these data representing climatic conditions most closely related to that of the Northern Territory (latitude of <30ºS during summer-autumn) revealed that over 35% of males and almost 60% of females were insufficient (<75nmol/L)[23]. Another study performed in southeast Queensland (27°S) found 41% and 15% of women were deficient (<50nmol/L) in winter-spring and summer respectively[24]. Thus, vitamin D insufficiency appears less common in a tropical climate or during the summer, however, the lack of congruency in the data described above suggests that population-level factors, testing bias or study design might also influence reported 25OHD3 levels.

It has been recently demonstrated that routine LC-MS/MS methods can overestimate serum vitamin D levels by as much as 11% compared to reference LC-MS/MS methods, most likely because of failure to resolve the 25OHD3 epimer, 3-epi-25OHD3[25]. The RDDT LC-MS/MS method does not delineate the 25OHD3 epimers yet did correlate well with the commonly used RIA radioimmunoassay (DiaSorin) which does not bind 3-epi-25OHD3. It is therefore possible that our estimates of serum vitamin D insufficiency are conservative.

In our study, the point estimate for mean serum 25OHD3 levels was lower in Indigenous children though not statistically significant (78 versus 91nmol/L; p=0.063). Melanin content of the skin influences the number of photons that reach the lower cellular layers, where 25OHD3 synthesis takes place, and the amount of 25OHD3 produced following equivalent sunlight exposure is lower for darker skinned individuals[26]. Few studies have investigated 25OHD3 levels in Indigenous Australians. In temperate South Australia, the mean 25OHD3 level among 58 healthy Indigenous adults was 57nmol/L[27]; well below the cut-off for insufficiency (75nmol/L).

Although most children (64%) were breast fed at the time of hospitalisation, we found that those children who were not breast fed had significantly higher levels of 25OHD3. Stratification by Indigeneity highlighted that the effect was limited to the non-Indigenous non-breast fed sub-group where high 25OHD3 levels (102nmol/L) were found (Table 3). It is difficult to draw a conclusion regarding breast feeding and 25OHD3 levels because of the exclusive nature of the definition (breast feeding at hospitalisation) and the age difference between breast feeding and non-breast feeding children (5 versus 12 months). Age had little influence on vitamin D levels in this study but others have shown breast fed infants are at greater risk of vitamin D insufficiency even in sunny climates[14]. Greater food intake and/or the use of vitamin D fortified infant formulas among non-Indigenous non-breast feeding mothers and their children are plausible explanations.

Vitamin D has been postulated to be a potential panacea for a range of diseases including diabetes, cancer, heart, infectious and autoimmune diseases such as multiple sclerosis. However, most of these data come from association studies. A recent meta-

analysis found that vitamin D supplementation had a net protective effect against respiratory tract infections[13]. Importantly that analysis showed that age and baseline vitamin D status had no effect on the outcome but that daily dosage was significantly more effective than bolus dose supplementation.

Limitations

This was a small opportunistic cross-sectional study and results should be interpreted with caution. Only children hospitalised for predominantly infectious diseases and with blood drawn for pathology could be investigated and small numbers limited the ability to interpret the relationship between vitamin D and the less common diagnoses. Furthermore, as with any cross sectional study, there was uncertainty whether vitamin D insufficiency was a cause or an effect of the investigated diseases and recent evidence suggests vitamin D is a negative acute phase reactant that is depleted following an inflammatory insult[28].

Conclusions

This is one of the few reports on vitamin D among Indigenous Australians. Vitamin D insufficiency was evident in one third of these hospitalised children of the Northern Territory with the lowest vitamin D levels seen among children hospitalised with gastroenteritis. If vitamin D insufficiency does increase the risk of ALRI among Indigenous children in this setting, it will be important to conduct a larger population-

283 based study where children with vitamin D insufficiency can be compared against non-
284 hospitalised/healthy controls. The high level of exposure (% insufficiency) suggests that
285 either prospective or retrospective cohort studies are feasible options for the future.

286

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297

298 Competing interests

299 None.

Table 1. Reason for hospitalisation of study participants (n=74 children)

There were 124 ICD-10-AM[15] discharge diagnoses among the 74 children at an average of 1.7 diagnoses per child. There were 51 ICD-10-AM diagnoses among the 44 ALRI hospitalised children (*italics*) dominated by bronchiolitis (n=22) and pneumonia (n=21). ICD-10-AM codes were obtained from Royal Darwin Hospital by the medical records department.

Diagnosis	ICD-10-AM codes	Children	
		n	%
Acute lower respiratory infection	J09-J18.9, J20-J22, R05, J98.4 or A37.0	44	59
Bronchiolitis	J21-J21.9	22	30
Pneumonia	J12-J18.9	21	28
Non-specific ALRI	J20-J20.9, J22, J98.4 or R05	5	7
Confirmed influenza	J09	2	3
Pertussis	A37.0	1	1
Gastroenteritis	A00-A09, B79.0, R11.0	20	27
Upper respiratory tract infection	J00-J06.9, H66.3, H66.9, J72.9	14	19
Anaemia	D50-D53.9	13	18
Urinary tract infection	N39.0, P39.3	11	15
Pyoderma (skin sores)	L00-L08.9, L20.0-L30.0, L23.1	11	15

Febrile illness	R50-R50.9	5	7
Unspecified viral infection	B34-B34.9	3	4
Sepsis	A40-A41.9	1	1
Respiratory obstruction	T17-T17.9	1	1
Hypertrophic cardiomyopathy	I42.1	1	1

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Table 2. Mean serum 25OHD3 levels according to participant characteristics

Mean serum 25OHD3 levels are shown by the occurrence of each participant characteristic. n is the number of children with (Yes) and without (No) each characteristic and % is the proportion of the total (n=74). Weight, haemoglobin and gestational characteristic data were not available for all children: discharge weight (n=69), haemoglobin (n=73), preterm (n=60), birth-weight (n=54), maternal smoking (n=73). Weight for age and haemoglobin levels were evaluated in relation to WHO defined reference values. Comparisons were performed using the Student's T-test and a p-value of <0.05 was considered statistically significant (bold).

General characteristics	**Yes**			**No**			
at hospitalisation	**n**	**%**	**nmol/L**	**n**	**%**	**nmol/L**	**p-value**
Male	47	64	86	27	36	83	0.618
Indigenous	42	57	78	32	43	91	0.063
<6 months of age	34	46	81	40	54	87	0.366
Breast fed	47	64	78	27	36	93	**0.029**
Indigenous	32	43	79	10	14	78	0.985
Non-Indigenous	15	20	78	17	23	102	**0.009**
Living remote	39	53	78	35	47	90	0.074
Attending childcare	12	16	94	62	84	82	0.167
Wet season (Oct-Mar)	38	51	81	36	49	87	0.302
Severely underweight	5	7	66	64	93	84	0.179
Haemoglobin <110g/L	29	40	77	44	60	88	0.118
Hospital stay >5days	31	42	82	43	58	85	0.721
Gestational characteristics							

Preterm (<37weeks)	14	23	70	46	77	91	**0.013**
Low birth weight (<2500g)	12	22	75	42	78	87	0.188
Maternal smoking	25	34	83	48	66	84	0.85

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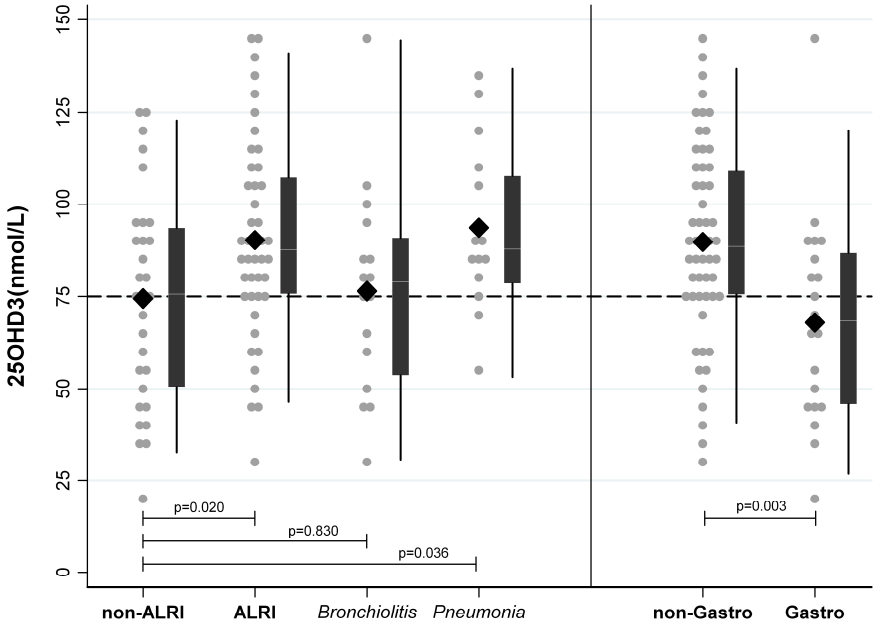
Table 3. Proportion of vitamin D insufficiency in children hospitalised with ALRI or gastroenteritis.

Proportions and odds ratios of vitamin D insufficiency (<75nmol/L) by ALRI (versus non-ALRI), exclusive bronchiolitis (versus non-ALRI), exclusive pneumonia (versus non-ALRI) and gastroenteritis (versus no gastroenteritis). Four of the 6 children concurrently diagnosed with ALRI and gastroenteritis had 25OHD3 levels below 75nmol/L. P-values were calculated using Fisher's exact test.

Hospital diagnosis		Totals	25OHD3 (<75nmol/L) n	%	Odds Ratio	95%CI	p-value
ALRI	Yes	44	10	23	0.34	0.11-1.03	**0.043**
	No	30	14	47			
Exclusive Bronchiolitis	Yes	16	7	44	0.89	0.22-3.55	1.00
	non-ALRI	30	14	47			
Exclusive Pneumonia	Yes	14	2	14	0.12	0.02-1.13	**0.049**
	non-ALRI	30	14	47			
Gastroenteritis	Yes	20	12	60	5.25	1.53-18.30	**0.004**
	No	54	12	22			

Figure 1. Vitamin D levels in children hospitalised with an ALRI or gastroenteritis.

Mean serum 25OHD3 was significantly higher in children hospitalised with an ALRI diagnosis compared to all other diagnoses. Mean serum 25OHD3 levels associated with an exclusive bronchiolitis diagnosis were comparable to the non-ALRI diagnoses whereas levels associated with an exclusive pneumonia diagnosis were similar to levels in the ALRI group overall. Mean serum 25OHD3 was significantly lower for the major non-ALRI diagnosis, gastroenteritis, compared to all other diagnoses. Six children had concurrent gastroenteritis and ALRI diagnoses. Grey dots represent the individual 25OHD3 levels and black diamonds identify the mean. Box and whisker plots identify the median (white line) interquartile range (black box) and 95% confidence intervals (lines). P-values were calculated using the Student's T-test.



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