RESEARCH PAPER

Vitamin D Supplementation for Treatment and Prevention of Pneumonia in Under-five Children: *A Randomized Double-blind Placebo Controlled Trial*

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Received: June 21, 2016; Initial review: July 25, 2016; Accepted: August 11, 2016.

Objective: To evaluate the efficacy of single oral mega-dose of Vitamin D3 for treatment and prevention of pneumonia in under-five children.

Design: Randomized, double blind, placebo-controlled trial.

Setting: Tertiary-care hospital.

Participants: 324 children (of 980 assessed) between 6 mo-5 y age (median (IQR): 12 (7,19.8) mo) with WHO-defined severe pneumonia. Of these, 126 (39%) were vitamin D deficient (serum 25(OH)D <12 ng/mL).

Intervention: 100,000 IU of oral cholecalciferol (*n*= 162) or placebo (*n*= 162) in single dose, administered at enrolment.

Outcome variables: *Primary*: Time to resolution of severe pneumonia and proportion of children having recurrence of pneumonia in next 6 months; *Secondary*: Change in serum levels of 25(OH)D; immunoglobulins IgA, IgG, IgM, and cathelicidin 2 weeks following supplementation; and time taken for overall resolution of illness.

Results: Median (95% CI) time for resolution of severe pneumonia was 30 (29, 31) h in the vitamin D group as compared to 31 (29,33) h in the placebo group [adjusted hazard ratio (95% CI): 1·39 (1·11, 1·76); *P*=0·005]. The risk of recurrence of pneumonia in next 6 months was comparable in the two groups [placebo: 36/158 (22·8%); vitamin D: 39/156 (25%); RR (95% CI): 1·13 (0·67,1·90); *P*=0·69]. Proportion of vitamin D deficient children declined from 38% to 4% in the supplementation group, and from 41% to 33% in the placebo group, two weeks after supplementation. There was no significant effect of vitamin D supplementation on serum levels of cathelicidin, IgA and IgG. The time taken for complete recovery from pneumonia, duration of hospitalization, and fever clearance time were comparable for the two groups. No adverse event was noted related to the intervention.

Conclusion: There is no robust evidence of a definite biological benefit, either for therapy or prevention, to suggest a routine megadose supplement of vitamin D3 for under-five children with severe pneumonia.

Keywords: *Cholecalciferol, LRTI, Micronutrient Therapy, Prevention, Outcome.*

Trial Registration: CTRI/2013/01/003317

Published online: August 30, 2016. PII:S097475591600022

Pneumonia remains the leading cause of childhood mortality accounting for 15% of all deaths in children below 5 years of age [1]. Observational studies have shown an association between vitamin D deficiency and respiratory tract infections, probably due to its immune-enhancing properties [2-13]. However, results of only few trials are available to document the efficacy of vitamin D supplementation on the incidence, severity, and recurrence of acute respiratory tract infections in under-five children [14-16]. Maneski-Holland, *et al*. [14] observed that administration of 100,000 IU of vitamin D to children (1–36 mo) with pneumonia made no difference to recovery but reduced the risk of repeat episode within 90 days. Another study [15] by the same group investigated the effect of 100,000 IU vitamin D_3 given orally once every 3 months for 18 months to healthy infants aged 1–11 months, and reported no significant difference between the incidence of first or only episode of radiologically confirmed pneumonia between the study and control groups. Another trial, conducted in India [16] by our group, evaluated oral vitamin D (1000 IU <1 year, 2000 IU >1 year) for 5 days to children 1–5 years with a clinical diagnosis of severe pneumonia and observed no difference in time to resolution of severe pneumonia, between the groups. A head on comparison or meta-analysis of these trials, though attempted [17], has limited validity because of substantial variability in the vitamin D

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dosing, definition of pneumonia, outcome measures, and duration of follow-up between various trials. Further, none of these trials documented the vitamin D level and immune status of the participants, either at baseline or after supplementation.

We planned to study the role of a single mega dose (100,000 IU) of oral vitamin D supplementation for treatment and prevention of community-acquired pneumonia along with estimation of baseline and post intervention serum 25(OH)D levels, and certain immune markers. The primary objectives were to document its effect on time to resolution of severe pneumonia and recurrence of pneumonia in next six months.

METHODS

Study design: This was a randomized, double blind placebo controlled trial conducted at a tertiary care hospital in New Delhi, India. Approval was obtained from the institutional ethical committee of the University College of Medical Sciences, Delhi. Informed written consent was taken from the caregivers.

Participants: Children aged 6 months to 5 years with a clinical diagnosis of severe pneumonia (defined as presence of lower chest indrawing in children presenting with cough or difficult breathing) [18] were included in the study. It was ensured that the family was staying within a 10 km radius of the hospital. Children having a history or clinical features suggestive of rickets (presence of wide wrists, delayed closure of anterior fontanel, presence of rachitic rosary, bow legs or knock knee), severe acute malnutrition, asthma, hypertension, complicated pneumonia (lung abscess, pleural effusion, empyema) or illness severe enough to require ventilation, chronic respiratory disease, heart disease, renal or hepatic insufficiency, neurological illness resulting in abnormalities of muscle tone/power, and known immunodeficiency were excluded. Children having received vitamin D or calcium supplements within four weeks prior to enrolment, those diagnosed with hypercalcemia or allergy to vitamin D, or immunized with pneumococcal/flu vaccine were also excluded.

Randomization and masking: Eligible children were randomized using computer-generated block randomization to receive 100,000 IU of vitamin D (cholecalciferol) or placebo orally. Eight, ten, and twelve blocks consisting of 10, 10, and 12 subjects, respectively were created. The drug and placebo were manufactured and supplied by M/s Zuventus Healthcare Ltd., India, in granule form, packed as 60,000 IU per sachet. The amount of vitamin D in the supplement was not determined independently from that described on the sachet. Both drug and placebo were identical in appearance, color, odor, amount, and taste. Five sachets of the drug were weighed and repackaged into three airtight zip pouch containing 100,000 IU of cholecalciferol each with the help of electronic weighing scale (0·001 g calibration). Placebo was also processed in similar manner. Only 15 doses were prepared at a time. Both drug and placebo were stored in a cool, dry, and dark place till dispensed. The next lot was prepared afresh when 4 doses were left. The allocation was further concealed by using sealed opaque envelopes. Randomization, repackaging, sequencing, and allocation concealment were done independently by a bio-statistician and an office secretary who were not members of the investigating team. None of the investigators, study staff, and participants was aware of the drug or placebo being dispensed. The codes were revealed only at the time of final data analysis.

Baseline data-collection: Details were recorded for socio-demographic variables (age, sex, socio-economic status, feeding practices), immunization status, nature and duration of presenting symptoms, and past history of similar episodes/nebulization. All children were examined for vital signs (temperature, heart rate, respiratory rate, blood pressure, oxygen saturation), pallor, cyanosis, nasal flaring, grunt, and mental status. Respiratory rate was measured for a full minute and if fast (RR >50/min for 6 months–1 year and >40/min for 1–5 years) [18,19], it was measured again and the two readings were averaged. The count was done at a time when the child was quiet. Axillary temperature was measured using a standard mercury thermometer. Fever was defined as temperature ≥38°C. Baseline oxygen saturation was measured using a pulse oximeter with a probe on a finger or toe, in room air. Chest was auscultated for presence of any added sounds (wheeze and/or crepitations). Weight, length/height, mid-upper arm circumference, and head circumference were recorded for all participants as per standard techniques [20]. Weight-for-age *Z*-score (WAZ), height/length-for-age *Z*-score (HAZ), and weight-for-height/length *Z*-score (WHZ) were derived using WHO Anthro software [21]. This software uses WHO reference standards for growth of under-5 children [22].

Intervention: A single dose of 100,000 IU of vitamin D (cholecalciferol) was dissolved in milk and administered orally or by nasogastric tube to the participant, on the day of enrolment after collection of the blood samples. Participants were treated as per a standard protocol. On admission, measures were taken to establish and maintain a patent airway, breathing, and circulation. Hydration was maintained and intravenous fluids were administered if

oral intake was poor. Oxygen was provided with a face mask or oxygen hood, if the child was having marked respiratory distress, signs of hypoxia, or oxygen desaturation. The child was nebulized with salbutamol if there was evidence of bronchospasm (presence of wheeze) or fast breathing with past history of nebulization. Blood samples were drawn only after the initial stabilization and before administration of drug/ placebo. Antibiotics were administered for severe pneumonia as per the guidelines of the Indian Academy of Pediatrics [23].

Hospital follow-up: Children were monitored and recorded every eight hourly for respiratory rate, chest indrawing, oxygen saturation, auscultation findings, fever, feeding, cyanosis, and mental status. Child was re-classified from severe pneumonia to pneumonia when lower chest indrawing disappeared (whereas fast breathing persisted), and remained absent for next 24 hours. The child was discharged when fever and fast breathing were absent for at least 24 hours.

Home follow-up: At home, participants were followed for 180 days (from day of enrolment) to document the recurrence of episodes of pneumonia. Field workers made home visits every fortnight starting from the day of discharge and enquired about episodes of cough or/and difficult breathing. An episode of cough associated with fast/difficult breathing (as reported by the mother) which warranted medical attention was regarded as an episode of pneumonia. The severity of pneumonia was not graded as the child was assessed by the field worker only at home. Wherever available, the records of hospitalization/ treatment were reviewed. An episode was regarded as 'recurrence' if the child remained free of symptoms of cough or fast breathing for at least seven days following completion of the course of antibiotic therapy as per protocol for the previous episode of pneumonia.

Investigations: Baseline hemoglobin, total leucocyte count, platelet count, blood culture, and chest *X*-ray were performed in all subjects at enrolment, as part of routine work-up. A 3 mL venous blood sample was obtained in a serum separator vacutainer at enrolment, at 14 days, and 3 months after enrolment. Serum were separated and stored after labeling them appropriately at –20ºC in a freezer. Blood samples for 25-hydroxyvitamin D and parathyroid hormone (PTH) were collected and transported in ice. Serum vitamin D (25(OH)D), Parathormone, serum calcium, serum phosphorous, and serum alkaline phosphatase were estimated in all three samples, in all participants. Immunological markers (serum immuno-globulin IgA, IgG, IgM, and cathelicidin anti-microbial peptide (CAMP)) were estimated at enrolment and after 14 days; initially in all subjects, and later in alternate participant.

Laboratory procedures: Serum PTH and serum 25(OH)D were estimated by radioimmunoassay (RIA) using commercially available kit manufactured by Immunotech SAS, France (interassay variation: below or equal to 10·3%; intra-assay variation: below or equal to 7·7%; sensitivity: 2 pg/mL) and DiaSorin, USA (interassay variation: 11%; intra-assay variation: 12·5%; sensitivity: at or below 1·5 ng/mL), respectively. Cathelicidin anti-microbial peptide (CAMP) in serum was estimated using a standard commercial kit (Human LL-37, HK 321 Hycult Biotech, Netherlands (sensitivity: 0·1 ng/mL), based on sandwich enzyme immunoassay (ELISA), as per manufacturer's instructions. Serum immunoglobulins (IgA, IgG and IgM) were measured quantitatively with immunoenzymatic colorimetric method using ELISA based kits (Xema Co Ltd, Russia) having a sensitivity of 0·12 g/L.

Outcomes: The primary outcome variables were (*a*) the time to resolution of severe pneumonia (the duration from the enrolment till the chest indrawing was no longer present, and continued to be absent for next 24 hours); and (*b*) the proportion of children having a recurrence of pneumonia in next six months. The secondary outcome variables included change in the serum level of 25(OH)D and PTH after two weeks and three months of therapy; change in serum level of cathelicidin and immuno-globulins (IgA, IgG, IgM) after two weeks of therapy; duration of hospitalization; time to complete recovery from pneumonia (normalization of respiratory rate), fever clearance time; and incidence rate of pneumonia during follow-up. All primary and secondary outcome measures were also studied in vitamin D deficient participants (serum 25(OH)D <12 ng/mL) [24].

Safety and adverse events: All recruited patients were assessed for clinical evidence of vitamin D intoxication in the first week after administering drug/placebo. Symptoms pertaining to hypervitaminosis such as dehydration, vomiting, decreased appetite (anorexia), irritability, constipation, fatigue, abdominal cramps, muscle weakness, and polyuria were enquired. Blood pressure was measured routinely for any evidence of hypertension. Biochemically, participants were monitored for presence of hypercalcemia (serum calcium greater than 10·8 mg/dL) [25] at two weeks as a sign of toxicity. Serum calcium above 14 mg/dL was set as the cut-off for treatment with intravenous furosemide and pamidronate (bisphosphonates) [26]. All adverse events occurring during 6 months follow-up period were also recorded.

STATISTICAL ANALYSIS

Sample size: Sample size was calculated for both primary outcomes, using data from a study by Manaseki-Holland, *et al*. [14] A sample size of 104 children in each group was considered adequate to detect a difference of 24 hours in time to resolution of severe pneumonia between the vitamin D (SD 2·22) and placebo (SD 2·89) groups, with 80% power and α = 0·05. To account for 10% attrition, the minimum required sample size was 115 for each group. For the second outcome measure, a sample size of 162 children in each group was required to detect a 30% relative reduction in proportion of children suffering from a repeat episode of pneumonia in the next six months in the vitamin D supplemented group, accounting for 20% attrition.

Statistical methods: Cox proportional hazards regression model was constructed to create the time to event curves and estimate the hazard ratio (HR) with 95% confidence interval (95% CI) between the treated and control groups for time to resolution of severe pneumonia, and adjusted for co-variates. Hazard ratio >1 indicates the relative likelihood of disease resolution in treated *versus* control subjects at any given point in time. Covariates included age, sex, nutritional status (WHZ score), severity of illness (respiratory rate), and baseline serum 25(OH)D levels. Incidence of pneumonia during follow up was calculated by dividing the total number of new episodes of pneumonia by total time at risk, for all children in each group. Relative risk for incidence of recurrence of pneumonia (the second primary outcome variable) was compared between the groups.

Changes (pre-post) in biochemical and immunological markers between the groups were compared by unpaired Student *t* test. Parameters which did not follow a normal distribution were log-transformed. Non-parametric (Mann Whitney U) test was used to compare groups if the applied transformation did not result in normal distribution. Within group means at baseline and follow-up were compared with paired t-test; or Wilcoxon signed rank test, if the data were not normally distributed. Kaplan-Meier survival function plots were constructed to compare the median duration for time to complete recovery from pneumonia, fever clearance time, and duration of hospitalization, between the two groups (placebo and vitamin D supplemented) by using the log rank test. P<0·05 was considered as significant. Bonferroni correction was applied to keep the type 1 error as 5% in total. The above analyses were also conducted for the subgroup of vitamin D deficient participants.

The effect of vitamin D supplementation on outcome variables was analyzed on an intention-to-treat basis. The data were analyzed by using SPSS software version 20·0.

A data and safety monitoring board (DSMB) reviewed and evaluated the accumulated study data on yearly basis for participant safety, study conduct, data management, and progress.

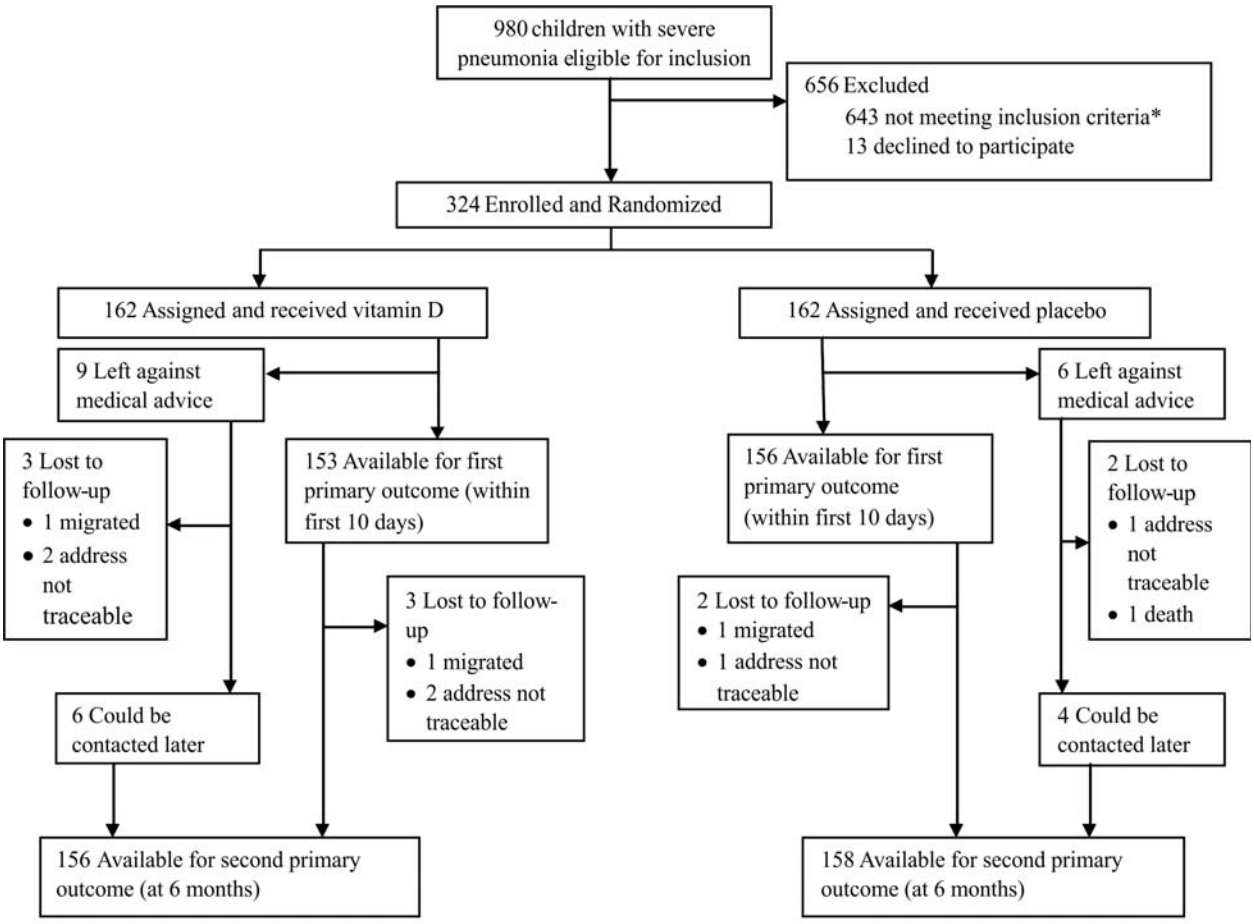
RESULTS

The participants were screened for recruitment between August 25, 2012 to January 27, 2015. Trial profile is depicted in ***Fig.* 1**. Randomized children (n=324) included 10 trial deviates (7 with rickets and 3 with heart disease) who were missed in the initial screening.

The median (IQR) age of enrolled children (226 (69·8%) boys) was 12 (7,20) months. Number of children in age categories of 6 m–1 y, 1–2 y, and >2 y were 186 (57·4%), 75 (23·2%), and 63 (19·4%), respectively. Of the 324 participants, 121 (37·3%) children had WHZ scores between –1 to –2 SD, followed by 89 (27·5%) participants in median to –1 SD. 69/324 (21·3%) participants had WHZ score between –2 to –3 SD. Similar trends, with maximum number of participants in –2 to –3 SD category, were observed for weight-for-age Z-score (118/324) and length-for-age Z-score (103/324). Baseline socio-demo-graphic and clinical characteristics of the participants in the two study arms are compared in ***Table* I**.

The prevalence of anemia (Hb <11 g/dL) in the study population was 267/324 (82·4%); hypocalcemia (calcium <8·8 mg/dL) and hypophosphatemia (serum phosphorus <3·8 mg/dL) were observed in 55·9% (180/322) and 31·4% (101/322) participants, respectively. Raised serum alkaline phosphatase (>283 IU/L for 1-12 months, >345 IU/L for 13-36 months, and >309 IU/L for > 37 months age) was documented in 47 participants. The baseline hematological, biochemical, hormonal and immunological parameters between the two groups are compared in ***Web Table* I**. Vitamin D deficiency (serum 25(OH)D <12 ng/mL) was present in 61/162 (37·6%) children in vitamin D supplemented group compared to 65/162 (40·1%) in the placebo group. Blood culture was positive in 28 (8·6%) children, of which *Staphylococcus aureus* was isolated in 27 cases. Baseline chest *X*-ray was abnormal in 292 (90·1%) children. Consolidation or bilateral patchy opacities were observed in 14 children, while the rest had hyperinflation and/or minor infiltrates.

Web Fig.* 1*a shows resolution of severe pneumonia as survival curves for the two groups, after adjusting for covariates. Median time taken for resolution of severe pneumonia was 30 (95% CI 29, 31) h in the vitamin D group as compared to 31 (95% CI 29, 33) h in the placebo



*Reasons for exclusion (*n*): residence more than 10 km away from the hospital (53); received vitamin D or calcium supplements (22); weight for height Z-score <-3 (35); rickets (62); history of nebulization on 3 or more occasions (191); congenital heart disease (84); chronic respiratory disease (tuberculosis) (17); neurological illness: (46); renal/hepatic insufficiency (6); anemia requiring transfusion: (9); complicated pneumonia (55); severe illness requiring ventilator care (63).

FIG.1 *Trial profile showing participant enrolment.*

group. The unadjusted hazard ratio for resolution of severe pneumonia in treated *vs* control subjects at any given point of time was 1·31 (95% CI 1·04, 1·64; *P*=0·020). The difference was further adjusted for age, sex, respiratory rate at enrolment (for severity of illness), weight-for-height *Z*-score (nutritional status), and serum 25(OH)D levels. The relative likelihood of resolution of severe pneumonia in vitamin D supplemented group remained significantly higher after adjusting for respiratory rate and the rest of covariates (adjusted hazard ratio: 1·39 (95% CI 1·11,1·76; *P*=0·005). This translated to a 58% (95% CI 52–64%) chance of the patient having earlier resolution of severe pneumonia.

The proportion of children with recurrence of pneumonia in 6 months following supplementation and the number of children having multiple (>1) episodes of recurrence of pneumonia was comparable in the two groups (***Table* II**). The risk of a repeat episode of pneumonia within 6 months of supplementation was comparable between the two groups (placebo: 36/158 (22·8%); vitamin D: 39/156 (25%); relative risk: 1·13 (95% CI 0·67–1·90; *P*=0·69). The incidence of recurrence of pneumonia for children having received vitamin D was 0·056 episodes per month; compared to 0·052 episodes per month for children in the placebo group.

***Table* III** compares the secondary outcome measures between the two study groups. Number of vitamin D deficient children in the vitamin D supplemented group declined from 61/162 (37·6%) to 6/151 (4%), and 15/144 (10·4%), after 2 weeks and 3 months of follow-up, respectively. In the placebo group, the corresponding

TABLE I BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS (*N*= 324)

Variable	*Vitamin D supplemented group (N=162)*	*Placebo group (N=162)*
Age (mo), mean (SD)	16·4 (12·9)	16·9 (13·4)
Male: Female	113:49	113:49
Socioeconomic class, *n* (%)		
• Upper	3 (1·9)	2 (1·2)
• Middle	51 (31·5)	39 (24·0)
• Upper lower	108 (66·7)	121 (74·7)
Received exclusive breastfeeding for 6 months *n*, (%)	98 (60·5)	99 (61·1)
Total duration of breastfeeding (months) [Mean (SD)]	11 (7·0)	10 (6·7)
History of similar episode in past/nebulization [*n* (%)]	70 (43·2)	59 (36·4)
Children unimmunized *n* (%)	14 (8·6)	11 (6·8)
Nutritional Status		
• Weight (kg)	8·5 (2·38)	8·6 (2·60)
• Weight for age *Z*-score (WAZ)	–1·7 (0·95)	–1·6 (1·01)
• Height for age *Z*-score (HAZ)	–1·4 (1·19)	–1·5 (1·14)
• Weight for height *Z*-score (WHZ)	–1·2 (1·11)	–1·1 (1·07)
• Body mass index	15·2 (1·39)	15·3 (1·54)
• Mid upper arm circumference (cm)	13·3 (1·10)	13·4 (1·1)
Duration of illness (d), mean (SD)		
• Fever	3 (1·6)	3 (1·6)
• Breathlessness	1 (0·9)	1·4 (0·8)
Vital signs, mean (SD)		
• Respiratory rate (per minute)	62 (8)	62 (8)
• Oxygen saturation (%)	96 (3)	96 (3)
Physical signs, *n* (%)		
• Pallor	41 (25·3)	43 (26·5)
• Nasal flaring	115 (71·0)	114 (70·4)
• Grunt	1 (0·6)	0 (0)
• Wheeze	137 (84·6)	127 (78·4)
• Crepitations	158 (97·5)	162(98·8)
Clinical diagnosis on admission, *n* (%)		
• Bronchopneumonia	98 (60·5)	100 (61·7)
• Bronchiolitis	22 (13·6)	21 (13·0)
• Wheeze associated respiratory tract infection	42 (25·7)	41 (25·3)

proportion of vitamin D deficiency was 40·1%, 33·3%, and 36·2%, respectively at baseline, 2 weeks, and 3 months. Duration of hospitalization, time taken for recovery from pneumonia, and time taken for resolution of fever were comparable between the two groups (***Web Fig.*1b-d**).

Only 9/324 (2·8%) children required re-dosing of the supplementation (6 in placebo group and 3 in vitamin D group). Of these, five children (four in placebo and one in vitamin D group) had a single episode of vomiting immediately after ingestion and rest of the children spilled the content.

No adverse reaction were noted in any of the study participants following supplementation. Hypercalcemia (serum calcium greater than 10·8 mg/dL) at 2 weeks was not noted in any of the participant.

Mean duration of follow-up for 318 participants (home address of 6 participants were not traceable) was 23·8 weeks. Numbers of adverse events during follow-up

TABLE II RECURRENCE OF PNEUMONIA IN 6 MONTHS FOLLOWING THE RESOLUTION OF THE INITIAL EPISODE

Variable	*Supplementation group*		*P value*
	Vitamin D (n=156)	*Placebo (n=158)*	
Recurrence of pneumonia, n (%)	39 (25)	36 (22.8)	0·64
1 episode	29	27	
2 episode	7	6	
3 episode	3	2	
4 episode	0	1	

in the two groups are compared in ***Table* IV**. All the adverse events happened after 4 weeks of administration of intervention. There was no death during hospitali-zation or within first 14 days of enrolment. One death was reported after 28 days of enrolment by the field staff. This child, belonging to the placebo group, left without information on day 2 of hospitalization. The field staff came to know that the child died 26 days later in the village.

We also compared the outcome measures for vitamin D deficient participants (serum 25(OH)D <12 ng/mL; *n*=126/324 (38·9%), in a sub-group analysis. For estimation of time to resolution of severe pneumonia, 116/126 (92·1%) vitamin D deficient participants were available (56/61 (91·8%) in vitamin D group and 60/65 (92·3%) in placebo group). Median time for resolution of severe pneumonia was 30 h (95% CI 27–33 h) in the vitamin D group as compared to 32 h (95% CI 24–40 h) in the placebo group (unadjusted hazard ratio 1·46 (95% CI 1·01, 2·12); *P*=0·047). The difference was adjusted for age, sex, nutritional status, respiratory rate and baseline serum 25(OH)D levels. The relative likelihood of resolution of severe pneumonia in vitamin D group remained significantly higher (adjusted HR: 1·66 (95% CI 1·12, 2·45); *P*=0·011). The risk of a repeat episode of pneumonia within 6 months of supplementation in vitamin D deficient children was comparable in the two groups (placebo: 14/63 (22·2%); vitamin D: 11/58 (19%); relative risk: 0·82 (95% CI 0·34-2.0); *P*= 0·82). Secondary outcome variables for vitamin D deficient children were also comparable between the two groups (data not shown).

DISCUSSION

In this randomized, double blind, placebo controlled trial, we observed that vitamin D (cholecalciferol, vitamin D_3) administered in a single oral dose of 100,000 IU to children aged 6 months to 5 years with severe pneumonia (as defined by WHO: any child with cough/fast breathing with lower chest indrawing), hastens the resolution of

TABLE III SECONDARY OUTCOMES IN THE TWO STUDY GROUPS

Secondary Outcomes	*Supplementation Group*				*P value*
	N	*Vitamin D*	*N*	*Placebo*	
Change in serum 25 (OH) vitamin D (ng/mL), mean (SD)					
From baseline to 2 weeks	151	30·1 (27·1)	144	1·9 (14·7)	<0·001
From baseline to 3 months	144	7·0 (18·8)	138	0·2 (16·9)	0·002
Change in serum PTH (pg/mL), mean (SD)					
From baseline to 2 weeks	151	–14·9 (53·1)	144	0·5 (42·3)	0·006
From baseline to 3 months	144	–8·2 (56·2)	138	–1·9 (59·0)	0·36
Change in serum cathelicidin (ng/mL), mean (SD)					
From baseline to 2 weeks	74	–1·5 (14·7)	76	1·6 (8·4)	0·12
Change in serum IgA (mg/dL), mean (SD)					
From baseline to 2 weeks	119	–0·1 (0·5)	122	–0·1 (0.4)	0·43
Change in serum IgG (mg/dL), mean (SD)					
From baseline to 2 weeks	119	0·6 (2·9)	122	0·2 (1·8)	0·26
Change in serum Ig M (mg/dL), mean (SD)					
From baseline to 2 weeks	119	0·3 (1·7)	122	0·4 (1·7)	0·92
Duration of hospitalization (h), mean (SD)	152	104·7 (37·9)	156	109·4 (46·0)	0·32
Time to complete recovery from pneumonia (h), mean (SD)	153	48·8 (25·0)	156	50·8 (29·5)	0·93*
Fever clearance time (h), mean (SD)	78	20·7 (18·2)	80	18·1 (14·1)	0·50*

*P value computed on means and *log transformed means by unpaired t-test.*

TABLE IV ADVERSE EVENTS DURING FOLLOW-UP OF 6 MONTHS

Adverse events	*Supplementation group*	
	Vitamin D (n=156)	*Placebo (n=159)*
Adverse events*, *n*(%)	54 (34.6)	49 (30.8)
One	45	42
Two	7	7
Three	2	0
Serious adverse events, *n*(%)	19 (12.2)	20 (12.6)
Serious adverse events other than pneumonia, *n* (%)	4 (2.6)	2 (1.3)

**Other than pneumonia and acute upper respiratory infections.*

lower chest indrawing by one hour which is statistically significant but may not be relevant, clinically. There was no significant reduction in the time taken for complete recovery from pneumonia, duration of hospitalization, and fever clearance time. Further, the supplementation did not prevent the recurrence of pneumonia in next six months. Similar observations were made in a subgroup analysis that included only vitamin D deficient children.

Lack of a wider therapeutic or preventive benefit of vitamin D supplementation in severe pneumonia was observed despite a significant improvement in serum 25(OH)D levels. Efficacy of vitamin D supplementation for improving the vitamin D status was evident from a significant reduction in proportion of vitamin D deficient children in the supplementation group. Vitamin D supplementation did not bring about any significant change in the serum levels of immunoglobulins IgA and IgG of study participants. A marginal increase occurred in serum IgM levels in children supplemented with vitamin D. Serum cathelicidin, an antimicrobial peptide, said to be responsible for the immunity enhancing property of vitamin D, remained unaffected after vitamin D supplementation.

We were unable to replicate the "effect" seen in the Kabul study [14] *i.e*., reducing the frequency of recurrence of pneumonia by vitamin D supplementation. The difference can be explained on the basis of certain parameters that were uneven between the two studies including the definition of pneumonia. We included all cases that satisfied WHO definition of severe pneumonia [18], including those with wheeze; whereas the Kabul study excluded all children with wheeze. More than 80% of our children had wheeze and could be either harboring a viral infection or having a component of allergic respiratory disorder. Studies have suggested that 1,25(OH)D may induce the production of immune markers only in presence of some specific pathogens [27,28] and probably those pathogens were in low numbers in our study. Secondly, the illness severity was more in our children since all our participants had severe pneumonia; Kabul study included children with both pneumonia and severe pneumonia. There is a possibility that in more severe cases, the conversion of 25(OH)D to 1,25(OH)D, which is the active metabolite, gets hampered [29]. It would have been very appropriate to also measure vitamin D status at 6 months, as 25OHD concentrations between the control and active groups were nearly similar at 3 months and would most probably have been no different at 6 months. This discussion does raise the question about whether or not the response might have been different if 100,000 IU had been given more frequently (eg. every three months). We could not compare our results on early resolution of severe pneumonia since the Kabul study was underpowered to comment on this outcome due to less number of children with severe pneumonia.

Our study had certain limitations. We included a heterogeneous group of conditions under the blanket diagnosis of severe pneumonia, because of the pitfalls of its definition. WHO criteria to identify severe pneumonia are highly sensitive but have a low specificity. Clinically, most of these children had a wheezy illness and very few had consolidation/bacterial pneumonia; a few may have been suffering from allergic respiratory illness. Moreover, we did not attempt a microbiological diagnosis of pneumonia by lung tap or bronchoalveolar lavage. Immune and clinical response to vitamin D may vary for bacterial/viral illness. While studying immune responses, we did not have any outcome measures related to cell-mediated immunity. Flow-cytometry and evaluation of cytokines could not be undertaken for want of logistics. We admit that our workup for parameters of humoral immunity was also not holistic. Finally, we were able to exclude clinical rickets at enrolment but not severe vitamin D deficiency based on 25OHD levels. No child developed clinical rickets during the course of the study. An ethical issue may not arise as there is no clear consensus about whether asymptomatic vitamin D deficiency needs to be treated.

To conclude, we did not find any robust evidence to recommend routine supplementation of vitamin D to children with severe pneumonia to hasten the resolution of illness or to prevent recurrence of further episodes in next 6 months. It however remains to be seen whether similar results are obtained in children with radiological pneumonia, and those belonging to other racial groups and nutritional status.

WHAT IS ALREADY KNOWN?

- Observational studies have shown an association between vitamin D deficiency and respiratory tract infections, probably due to its immune-enhancing properties

WHAT THIS STUDY ADDS?

- Vitamin D supplementation to children with pneumonia may improve the vitamin D status and offer a marginal benefit in reduction of disease severity and recurrence. However the advantage offered is neither clinically significant nor consistent to warrant routine supplementation of vitamin D in children below five years of age with pneumonia.

Acknowledgments: The trial was supported by funding from the Indian Council of Medical Research, New Delhi. The drug and placebo used in the study were provided by Zuventus Healthcare Ltd. India.

Contributors: The study was conceived by PG, building on a previous study by PG and NB. PG, PD, DS, IRK, AKB, and SVM contributed to study design and writing the proposal for research. Data collection was handled by NS, and supervised by PD, DS, and PG. IRK and SVM supervised the laboratory work-up of immune markers, and vitamin D status, respectively. Statistical analysis was carried by AKB and PG. Literature search was conducted by NB, NS, and PG. Initial draft of the manuscript was written by NB and NS which was edited and refined by PG. PD, DS, SVM, IKK, and AKB provided critical inputs to the draft manuscript. The manuscript was seen and approved by all authors.

Funding: Indian Council of Medical Research.

Competing interests: Zuventus Healthcare Ltd. India provided the drug/placebo and are manufacturers of vitamin D formulations. They, however, had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript.

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