Cover page

Study Protocol with statistical analysis plan (SAP)

Title: Randomized Controlled Trial of Vitamin D supplementation on Improvement of Pneumonic Children at Tertiary Pediatric Hospital in Egypt

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Introduction:

Globally, pneumonia is considered one of the prominent causes of children's morbidity and mortality, particularly in developing countries, causing 29% of the under-Five mortality**(1).** According to the World health organization estimates, 66% of deaths due to pneumonia occur in infancy, where 90% occur in developing countries**(2).**

In Egypt, according to UNICEF 2018, Acute Respiratory Tract Infection (ARTIs)was estimated to account for 11% and 19% of the under-five and post-neonatal mortalities respectively(3).

Despite the well-recognized role of vitamin D in metabolism and homeostasis in the general population, there is now growing interest in its probable association with pneumonia(4).

Basically, 25(OH) D and 1,25 (OH)2D are surrogate markers of the active form of vitamin D in the body. Recently, it has been highlighted that Vit D(1,25(OH)2D) has a crucial role in host defenses, inflammation, and immunity. Furthermore, Vit D (1,25(OH)2D) promotes the expression of cathelecidin and B-defensin (antimicrobial peptides) that are broadly expressed in the body and playa vital role in innate immunity as a result of their chemotactic action and toxin neutralization(5).

Due to its short half-life (12-36 hours) compared to 25(OH)D (3 weeks), 1,25(OH)2D is proposed to provide a more valid measure for better prediction of the vitamin D role in reducing the severity and improving the prognosis of patients with (ARTIs)(6).

Globally, about 30% to 90% of Under-5 children experience vitamin D deficiency. This could vary among children, according to the socioeconomic, environmental and behavioral circumstances.

Studies evaluating the association of 1,25 (OH)2D deficiency and the severity of respiratory tract infection, are rare and showed controversial findings(7,8).

However, an Indian systematic review polled the results of 12 studies, with 2279 participants, highlighted the significant correlation between vitamin D deficiency and incidence and severity of ALRIs(9).

A prospective cohort study conducted in Yemen in 2009, examined the ability of deficient levels of vit.D to predict the outcomes of severe pneumonia. The study documented the significant association between vitamin D deficiency with neutropenia and hypoxia in patients with severe pneumonia, thus predicting poor prognosis(10).

In Egypt 2010, a case-control study conducted on children aged 2 to 5 years to examine the impact of vitamin D deficiency on the susceptibility of pneumonia. The study illustrated that Vitamin D deficiency is associated with a higher incidence and more severe pneumonia(11).

Hashemian H., et al 2017, advocated providing children(particularly suffering from pneumonia) with adequate amounts of vitamin D supplements(12).

Nevertheless, few studies have been conducted to evaluate the impact of vitamin D supplementation on the outcome of pneumonic infants.

Thus, we urge to conduct a randomized controlled trial (RCT) in Abou ElReesh tertiary Pediatric hospital, to evaluate the effects of vitamin D3 supplementation to children with pneumonia. We postulated that supplementation of 100 000 IU of vitamin D3 (Cholecalciferol) will reduce the duration of illness in those children and improve their outcome.

Subjects and Methods:

Study design and study setting

The current study is a randomized, double-blinded placebo-controlled trial (RCT) that was conducted in Cairo University Abou ElReesh Children hospital, which is a university-affiliated teaching hospital in Egypt, to evaluate the effect of vitamin D3 supplementation on the outcome of treatment of children with pneumonia. Pneumonia cases were recruited from two of the hospital general pediatric departments and the four pediatric intensive care units (PICUs) of the hospital.

Sample Size and study population

To detect a 20% difference in the mean duration of pneumonia between the vitamin D arm and control arm [5 days vs. 6 days (SD 2)] allowing Type I error of 5% and Type II error of 10%, 86 per group (172 total) is the minimum number to be recruited [13]. After allowing for 10% for nonresponse or loss for follow-up and 25% for exclusion of children diagnosed with sufficient or toxic level of vitamin D after vitamin D testing (Box 1) according to prevalence of vitamin deficiency in a pediatric hospital of eastern India [14], 233 was the minimum number to start with. Mild and moderate pneumonia cases were recruited from two of the hospital general pediatric departments chosen by simple random sample from a total of six hospital departments while severe pneumonia cases were recruited from the four PICUs of the hospital.

All children (259 children) between 1 month and 12 years of age admitted to the selected departments during the period from 9th September 2019 to 15th December 2019 and diagnosed clinically with pneumonia according to World Health Organization criteria of severity [15] (Box 2) were screened for the inclusion criteria. Children who had clinical signs of rickets (2 children), severe illnesses (meningitis, heart or renal disorders, measles, severe malnutrition, endocrine dysfunction, hypercalcemia, hyperthyroidism, and suspected tuberculosis) (4 children) or were known to have received high-dose vitamin D treatment in the past 3 months (3 children) were excluded from the study. Children with sufficient (52 children) or toxic (7 children) levels of vitamin D were also excluded.

Thus, 191 child (93 in the intervention arm and 98 in the control arm) who had pneumonia with insufficient or deficient level of vitamin D and their parents consented to participate were actually enrolled in the study, completed the baseline and the outcome assessments and continued the daily clinical follow up till their discharge (improvement or death) from the hospital.

Box 1: Level of vitamin D [16**]**

Sufficient vitamin D (25(OH)D: ≥ 30 ng/mL),

Insufficient vitamin D (25(OH)D or at risk of deficiency : 10-29 ng/mL), and

Deficient vitamin D (25(OH)D: <10 ng/ mL).

Box 2: Diagnosis of Pneumonia [15**]**

Pneumonia: (i) Age-specific tachypnoea (>60/min if <2 months; >50/min if 2–11 months; >40 if 12–24 months) and (ii) absence of wheeze (with or without fever).

Fever: Axillary temperature >37.50 _C (age 1 week– 3 months) or >38.0 _C (2–23 months).

axillary temperature was measured using a standard mercury thermometers

Respiratory rate was measured twice for one full minute while the child is quiet using stopwatches taking the average count.

Mild pneumonia: Minimally increased work of breathing, no hypoxemia, able to tolerate PO

Moderate pneumonia: Hypoxemia, inability to tolerate **PO, moderately** increased work of breathing (grunting, retracting, tachypnea).

Severe Pneumonia: Significantly increased work of breathing, altered mental state, concern for respiratory failure sepsis, failure to maintain oxygen saturation with Fi02 of 50%, need for positive pressure ventilation. Oxygen saturation was measured using a pulse oximeter with a probe on a finger or toe, in room air.

Improvement (discharge criteria): (Meets all) tolerating PO, not hypoxemic (> 90%), mildly increase or normal work of breathing [17].

Vitamin D testing

Two milliliters of venous blood was collected on plain tubes, left for 10 min to clot and then centrifuged at 3000 rpm for 5 min; the separated serum samples were stored at -20 °C till the time of assay and used for detection of concentrations of serum 25OH Vitamin D Total (25OH Vitamin D2 and D3) by ELISA using (DIAsource 25OH Vitamin D Total ELISA Kit, Catalogue No. KAP 1971) supplied by DIA source Immunoassays S.A. (Rue Du Bosquet, 2 B-1348 Louvain-la Neuve, Belgium), according to the manufacturer's instructions and based on the principle of solid-phase Enzyme-Linked Immunosorbent Assay performed on microtiter plates. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the total 25OH Vitamin D (D2 and D3) concentration. A calibration curve is plotted and the total 25OH Vitamin D (D2 and D3) concentrations of the samples are determined by dose interpolation from the calibration curve.

Random allocation of study groups

The children were individually randomized into intervention or control groups using a random number sequence elicited in an Excel spreadsheet with no limitations. The allocation was further hidden by using closed dark envelopes. A biostatistician and an office secretary who were not members of the investigating team did independently the randomization, repackaging, sequencing, and allocation concealment. None of the study staff and participants' parents was aware of the drug or placebo being dispensed. The codes were revealed only at the time of final data analysis.
The children were followed clinically on a daily base by the three study pediatricians to assess the resolution or deterioration of signs and symptoms of pneumonia till being discharged from the hospital whether due to recovery or death. The routine treatment of inpatient children is free of charge in Abou ElReesh Children's hospital, while vitamin D was paid by the researchers to be given free of charge to the children in the intervention arm during the study period.

Baseline assessment

Data was collected for each participant regarding socio-demographic variables (age, sex, feeding practices), skin color, nature and duration of presenting symptoms. All children were examined for vital signs (temperature, heart rate, respiratory rate, blood pressure, oxygen saturation), pallor, cyanosis, nasal flaring, grunt, and mental status. Pa O2/FIO2 was measured and serum creatinine, C reactive protein, platelet count and serum bilirubin laboratory tests were done. SOFA score was assessed for severe pneumonia cases.

Intervention

All children were treated with antibiotics according to WHO classification and treatment of childhood pneumonia at health facilities 2012 [18], at enrollment after obtaining consent from parents and completing the baseline assessment, children were given a single injection of one ml of 100.000 IU of vitamin D3 (Cholecalciferol), vitamin D3 obtained from 2 ml vials containing 200,000 IU each (Devarol- S- 200.000 I.U. produced by Memphis for Pharmaceutical and Chemical Industries) and stored in manufacturer's recommended conditions in a dry, cool environment for 1–16 weeks (depending on the date of recruitment) or placebo which is 1 ml saline injection. Syringes were labeled with a unique ID number and given by the blinded doctors choosing the next syringe with a randomization code. (only office secretary aware of randomization codes).

Hospital follow-up

Children were monitored with recording data every eight hours for respiratory rate, chest indrawing, oxygen saturation, auscultation findings, fever, feeding, cyanosis, and mental status. The child was discharged when fever and fast breathing were absent for 2 consecutive days.

Outcomes assessment

Outcomes were assessed 7 days after vitamin D injection, the first day whereas vitamin D reaches its maximum level in the blood [19] to guarantee the assessment of all participants before their

discharge. The primary outcome variables were changed in serum level of 25(OH)D, PaO2/FIO2, serum creatinine, C reactive protein, and serum bilirubin levels and platelets count. Also, the SOFA score for severe cases. The secondary outcomes included the fate of the cases (improvement or death) and the duration between enrolment and hospital discharge for improved cases.

Statistical analysis:

Patients' percent change of Vitamin D (25 (OH)2D) was calculated to quantify the change levels before and after vitamin D3 (Cholecalciferol) administration through the equation:

Pediatric, Blood Vitamin D level (BVD) %change =

[(BVD after – BVD before) ÷ BVD before] × 100

Collected data were entered and analyzed using the Statistical Package for Social Science Software (SPSS) program, version 21.0 IBM. Tests of normality of data (like Shapero-Wilk test) revealed that data isn't normally distributed. That's why non-parametric tests like MannWhitney and Kruskal-Wallis tests were used in univariable comparisons to quantify the associations of continuous variables. Data were summarized using the median and interquartile range for quantitative variables. Spearman correlation test was used to detect the relationship between continuous variables. P values below 0.05 were considered statistically significant. A multivariate Cox regression analysis model was conducted to explore the independent effect of vitamin D3 supplementation on the overall survival of pneumonic children.

Given the short period of the study follow-up and single intervention, there was no stopping rule or interim analyses.

All children randomized were included in the analysis on an intention-to-treat basis unless the outcome measures were missing (n = 0) or reported to have recovered or lost within 24 h (n = 0). Meantime to recovery for the episode of pneumonia at recruitment was compared for the vitamin D group and control group. Kaplan–Meier plots and log-rank tests were used to compare the time to recover from the index episode of pneumonia between the vitamin D and placebo groups.

Ethical consideration

The study protocol and the consent form have been reviewed and approved on July, 2019 before study implementation by the department of public health and community medicine and pediatrics at Kasr Alaini faculty of medicine. The study protocol was also approved by Cairo University Ethical committee. Written consent was obtained from one of the child's parents if the child met the study criteria after explaining the study objectives, the nature of the intervention, risk and benefits from participating in the study and alternatives in case of participation refusal.

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