

## **Impact of Serum Vitamin D Status on the Outcome of Ventilator-Associated Pneumonia in Neonates**

### **Abstract**

**Objectives:** The aim of this work was to study the status of serum vitamin D level on the outcome of ventilator associated pneumonia (VAP) in neonates.

**Methods:** This prospective cohort study was carried out on 60 intubated neonates with gestational age > 32 weeks and ventilated for more than 48 h and included two groups, VAP group (n=30) and non-VAP group(n=30). Serum 25-hydroxy Vit D was tested at the start of mechanical ventilation, while blood culture and endotracheal culture were obtained after 2 days of mechanical ventilation and when VAP was suspected.

**Results:** The mean serum level of vitamin D in VAP group was statistically significantly lower than non-VAP group. Also, the total duration of mechanical ventilation, duration of O<sub>2</sub> supplementation post extubation and duration of hospital stay were statistically significantly longer in VAP group. Although the mortality was higher in neonates developed VAP, it didn't achieve a statistically significant difference. A cut off value of  $\leq 17.35$  ng/ml of serum 25-hydroxy vitamin D showed a sensitivity of 83.33%, specificity of 100 % and area under curve (AUC) was 0.895 to predict neonatal VAP

**Conclusion:** 25-hydroxy vitamin D deficiency is an important risk factor for VAP development in neonates, and low vitamin D levels is associated with significant longer duration of mechanical ventilation, post extubation O<sub>2</sub> support and hospital stay in neonates with VAP.

**Keywords:** Vitamin D, Ventilator Associated Pneumonia, Neonates, Outcome.

## **Introduction**

“Neonatal ventilator-associated pneumonia (VAP) is defined as a nosocomial lower airway infection in intubated newborns with onset beyond 48 hours of invasive mechanical ventilation” [1]. “Intubation and mechanical ventilation are known risk factors for the acquisition of nosocomial pneumonia” [2]. “VAP is one of the most frequently diagnosed nosocomial infections” [3] and “second most common cause for antibiotic use in neonatal intensive care units (NICUs) after early onset sepsis” [4,5].

“VAP is a common, serious and a real public health problem. It is associated with higher incidence of bronchopulmonary dysplasia, prolonged mechanical ventilation and NICU stay” [6].

“Vitamin D is a steroid hormone that has an important role in calcium and phosphorus homeostasis, bone metabolism and bone development” [7]. “Multiple reports suggested the vital role of vitamin D in immune system function and regulation since 1,25 dihydroxy vitamin D can promote the innate immature response to the pathogen” [8]. “Besides, many studies have identified an association of respiratory infectious diseases and inadequate serum vitamin D” [9, 10].

This study was conducted to detect the association between serum 25-hydroxy vitamin D<sub>3</sub> level and VAP development in intubated neonates.

## **Material and Methods**

This prospective cohort study was carried out at NICU of Tanta University Hospital on 60 neonates with gestational age > 32 weeks and ventilated for more than 48 h, from February 2021 to February 2022. Neonates with congenital pneumonia, multiple congenital anomalies, multiple organ failures, and requiring ventilation less than 48 h were excluded.

Patients were divided into two groups, **group A** (VAP group) included 30 neonates who developed VAP and diagnosed on basis of criteria suggested by pediatric modification of Center for Disease Control and prevention [11]. The diagnostic criteria included: inpatient without Iry underlying pneumonia; Chest x-ray showing newly progressive or persistent

infiltrate, consolidation and/or pneumatoceles; worsening of gas exchange with increasing  $FiO_2$  requirement and increasing oxygenation index. In addition to three of the following: Temperature instability without recognized cause; leucopenia, leukocytosis or left shift > 15%; new onset of purulent tracheal aspirate or increasing respiratory secretions with increasing suctioning requirement; apnea, tachypnea and retraction; wheezing and rales; changing in heart rates; clinical decision to change antibiotics and elevation in CRP > 10 mg/dl. **Group B (non-VAP group)** included 30 ventilated neonates with no VAP as a control group.

All neonates were ventilated by orotracheal tube which changed only if displaced or blocked, one set of disposable ventilator circuit was used for one patient and changed routinely every 3 days. Open suction was the method used and no topical oral antibiotics or antiseptics were used for cleaning the mucous membrane of the mouth. Serial Chest X ray were performed to all enrolled neonates. Complete blood count, CRP and serum 25-hydroxy Vit D were done with the start of mechanical ventilation, while blood culture and endotracheal culture were obtained after 2 days of mechanical ventilation and when VAP was suspected.

Newborns with VAP were subdivided into three groups according to their 25-hydroxy D levels, <5 ng/mL as severe deficiency group; 5–15 ng/mL as moderate deficiency group; and 15–30 ng/mL as mild deficiency group [12].

#### **1-Blood culture:**

Automated blood culture BacT/Alert (bioMérieux Inc., Hazelwood, MO) was performed using 2 mL of blood that were collected under complete aseptic procedure then placed directly into blood culture bottles. Bottles were incubated for five days before being discarded as negative and subculture according to the laboratory operating procedures if they flagged positively before this time [13].

#### **2-Endo-tracheal tube culture (ETT culture):**

Endotracheal secretions were collected by instilling 2 ml of sterile normal saline into the ETT and then collecting them back with the help of a sterile mucous trap, the specimen collected was immediately transported to the laboratory within one hour of collection. Endotracheal aspirates were subjected to bacteriological analysis. The aspirate was inoculated on blood agar media and MacConkey agar mannitol and sabaraux media then incubated for 24 hours at 37°C, colony characteristics were observed, and identification was done in accordance with standard recommendations. Sensitivity of organisms isolated was done by disc diffusion method by Kuby Bauer [14].

### **3- Serum 25-Hydroxy vitamin D:**

Serum 25-hydroxy vitamin D level was measured by ELISA (Enzyme-linked immune sorbent assay) technique. Venous blood was collected from each neonate by a sterile venipuncture and put in tubes labeled with the patient's name and his serial number. All tubes were centrifuged after they were allowed to clot at room temperature for separation of serum from whole blood. The serum was drained and put in clean dry tubes by means of clean dry pipette and stored at 2:8 °C [15].

### **Statistical analysis**

Statistical analysis was carried out using (SPSS 26.0, IBM/SPSS Inc.). Categorical data were described using numbers and percentages and compared by the  $\chi^2$  test (or Fisher's exact test). Quantitative data were described using mean and standard deviations (for normally distributed data) and median and inter-quartile range (for abnormally distributed data) were used.

To compare the two study groups with normally distributed quantitative variables, we used the independent samples t-test, and Mann–Whitney test if the data were abnormally distributed. The Kruskal–Wallis test used for abnormally distributed quantitative variables, while F-test (ANOVA) was used for normally distributed quantitative variables.

A receiver operating characteristic (ROC) curve was used to estimate the cutoff value of serum 25-hydroxy vitamin D in the prediction of VAP development. A p-value of less than 0.05 was considered statistically significant.

## Results

Demographic characteristics and clinical outcome of study groups were summarized in (Table 1). There was no significant difference between studied groups as regards gestational age, gender, birth weight, mode of delivery or antenatal risk factors. Maternal administration of calcium and vitamin D supplementation during pregnancy was significantly higher in non-VAP group than in VAP group, while CRP levels were significantly higher in neonates diagnosed with VAP.

The duration of invasive mechanical ventilation and duration of oxygen supplementation post extubation had a significant increase in VAP group in comparison to non-VAP group. Also, the duration of hospital stay was significantly longer in VAP group, but there was no significant difference in survival rate between both groups.

**Table 1: Demographic characteristics and clinical outcome of study groups**

	VAP Group (n=30)	Non-VAP group (n=30)	P-value
Gestational age (week)	34.37 ± 3.42	35.23 ± 2.92	0.29
Birth weight (kg)	2.25 ± 0.72	2.49 ± 0.81	0.24
Gender			
Male	18 (60%)	19 (63.3%)	0.79
female	12 (40%)	11 (36.7%)	
Delivery mode			
NVD	6 (20.0%)	9 (30.0%)	0.37
CS	24 (80.0%)	21 (70.0%)	
Antenatal risk factors			
Hypertension	6 (20%)	6 (20%)	1.000
DM	2 (6.7%)	2 (6.7%)	1.000
PROM	8 (26.7%)	2 (6.7%)	0.08
Maternal calcium or Vit. D intake	18(60%)	25 (83.3%)	0.045*
Haemoglobin (g/dl)	11.46 ± 1.69	12.20 ± 2.01	0.128
Platelets (×10 <sup>3</sup> /μl)	181.1 ± 102.8	227.7 ± 109.7	0.088
TLC (×10 <sup>3</sup> /μl)	13.86 ± 5.77	14.84 ± 6.90	0.739
I/T ratio	0.13 ± 0.08	0.11 ± 0.06	0.416
CRP (mg/L)	61.8 ± 36.15	17.43 ± 5.5	<0.001*

Na+ (mEq/L)	136.9 ± 6.12	139.23 ± 4.28	0.09
K+ (mEq/L)	4.28 ± 0.88	4.28 ± 0.76	0.98
Duration of mechanical ventilation (days)	23.7 ± 15.01	8.57 ± 3.96	<0.001*
Duration of O <sub>2</sub> support post-extubation (days)	6.5 ± 2.42	4.22 ± 1.24	0.001*
Duration of hospital stay (days)	34.7 ± 15.42	16.9 ± 4.66	<0.001*
Mortality	10 (33.3%)	7 (23.3%)	0.39

Data presented as mean ± SD, statistically significant at  $p \leq 0.05$ , CS: Caesarian section, NVD: Normal vaginal delivery, DM: Diabetes mellitus, VAP: Ventilator-associated pneumonia, PROM: Premature rupture of membranes, TLC: Total leucocytic count, I/T ratio: immature/total neutrophils, CRP: C-reactive protein

klebsiella and Staphylococci were the predominant pathogens isolated from blood cultures in neonates developed VAP, while in non-VAP group most of the blood cultures showed no growth and the difference between both groups was statistically significant. ETT culture results showed similar results, Klebsiella showed the highest growth in VAP group, while all ETT cultures showed no growth in non-VAP group (Table 2).

Serum 25-hydroxy vitamin D level was significantly lower in VAP group than non-VAP group ( $11.54 \pm 12.96$  versus  $34.99 \pm 10.1$  ng/ml respectively) and P-value was <0.001. All neonates in non-VAP group had serum vitamin D level > 15 ng/ml while in VAP group 19 neonates had vitamin D level < 5 ng/ml.

**Table 2: Blood culture, endotracheal tube ETT culture and serum 25-hydroxy vitamin D in studied groups**

	VAP Group (n=30)	Non-VAP group (n=30)	P-value
<b>Blood culture</b>			
No growth	9 (30.0%)	21 (70.0%)	<0.001*
Klebsiella	8 (26.7%)	1 (3.3%)	
Staphylococci	11(36.7%)	3 (10.0%)	
Streptococci	1 (3.3%)	2 (6.7%)	
Acinetobacter	1 (3.3%)	3 (10.0%)	
<b>ETT culture</b>			
No growth	0%	30(100%)	<0.001*
Klebsiella	22(73.3%)	0%	
Staphylococci	2(6.7%)	0%	
Streptococci	1(3.3%)	0%	
Acinetobacter	5(16.7%)	0%	
<b>25 (OH) Vitamin D (ng/ml)</b>	$11.54 \pm 12.96$	$34.99 \pm 10.1$	<0.001*
<5	19 (63.3%)	0%	<0.001*
(5-15)	6 (20%)	0%	
(15-30)	5 (16.7%)	12 (40%)	
>30	0%	18 (60%)	

Data presented as mean ± SD, statistically significant at  $p \leq 0.05$ , ETT: endotracheal tube, VAP: Ventilator-associated pneumonia.

In VAP group, when the patients were classified according to their vitamin D levels, there was significant decrease in total duration in mechanical ventilation in neonates with serum vitamin D level 15-30 ng/ml, while it was higher in neonates with serum vitamin D level < 15 ng/ml. There was no significant difference in terms of blood culture, ETT culture, survival rate or duration of hospital stay in VAP group with different degrees of vitamin D deficiency, but neonates with severe Vitamin D deficiency < 5 ng/ml showed longer hospital stay and higher mortality than other groups (Table 3).

**Table 3: Duration of mechanical ventilation, blood culture, endotracheal tube culture, survival rate and duration of hospital stays in VAP group**

	Vitamin D (ng/ml)			P
	<5 (n = 19)	5-15 (n = 6)	15-30 (n = 5)	
<b>Total Mechanical ventilation duration(d)</b>	37.67 ± 15.76	21.32 ± 13.91	16.0 ± 7.58	0.039
<b>Blood culture</b>				
No growth	7 (36.8%)	1 (16.7%)	1 (20%)	
Klebsiella	3 (15.8%)	3 (50%)	2 (40%)	
Staphylococcus	8 (42.1%)	2 (33.3%)	1(20%)	0.427
Streptococci	0 (0%)	0 (0%)	1 (20%)	
Acinetobacter	1 (5.3%)	0 (0%)	0 (0%)	
<b>ETT culture</b>				
No growth	0 (0%)	0 (.0)	0 (0.0)	
Klebsiella	14(73.7%)	5 (83.3)	3 (60)	
Staphylococcus	1 (5.3%)	1 (16.7)	0 (0%)	0.610
Streptococci	1 (5.3%)	0 (0%)	0 (0%)	
Acinetobacter	3 (15.8%)	0 (0%)	2 (40)	
<b>Survival rate</b>				
Survivors	11 (57.9%)	4 (66.7%)	5 (100%)	0.279
Non-Survivors	8 (42.1%)	2 (33.3%)	0 (0%)	
<b>Duration of hospital stay</b>	33.0	40.0	26.0	0.546

Data presented as median or frequency (%)χ<sup>2</sup>: Chi square test, \*: Statistically significant at p ≤ 0.05, ETT culture: Endo-tracheal tube culture, d: day

Our data showed that at cut off value of ≤17.35 ng/ml, serum 25-hydroxy vitamin D showed a sensitivity of 83.33%, specificity of 100 % and area under curve (AUC) was 0.895 to predict neonatal VAP (Fig. 1).

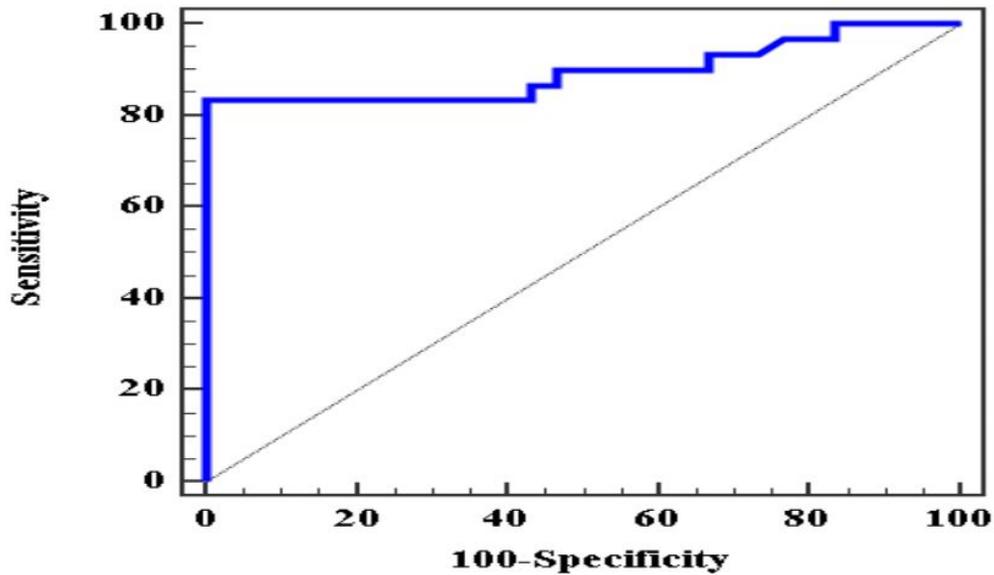


Figure 1: ROC curve for 25-hydroxy Vitamin D to predict neonatal VAP

#### Discussion

“In the current study, we investigated the association between neonatal VAP development and vitamin D levels and showed that vitamin D deficiency was a risk factor for VAP development. The mechanism by which vitamin D promotes immunity is complicated. It acts through the innate immune system by inducing antimicrobial peptides in epithelial cells, neutrophils and macrophages” [16]. Low levels of serum vitamin D may be significantly associated with neonatal pneumonia [8]. “Our data showed that serum level of vitamin D in VAP group was statistically significantly lower than in non-VAP group ( $11.54 \pm 12.96$  ng/ml and  $34.99 \pm 10.1$  ng/ml respectively). That was in line with the results of El-kassas et al. who reported that neonates with pneumonia showed significant lower levels of Vit. D compared to controls. Moreover, the authors showed that mechanically ventilated neonates revealed significant lower vit D levels compared to patients on free oxygen” [8].

Similarly, Lezhenko et al. [17] reported that “children at an early age with low levels of vitamin D are at risk for pneumonia since significant lower concentrations of 25-

hydroxyvitamin D were detected in children with community-acquired pneumonia compared with healthy controls”.

The hypothesis that lower serum vitamin D is a risk factor for neonatal pneumonia, and its poor prognosis can also be confirmed by the meta-analysis of Charan et al., [18] which stated that “vitamin D supplementation could decrease the events related to respiratory tract infections”. Additionally, the systematic review of Christensen et al.,[19] reported that “supplementation of vitamin D during pregnancy could prevent respiratory infections of offspring”. A recent study by Maretzke F., et al. found “a significant inverse association between vitamin D status and risk of acute respiratory tract infections, with randomized control trials supporting similar conclusion” [20].

Our study revealed that the duration of mechanical ventilation, post-extubation O<sub>2</sub> support and duration of hospital stay were statistically significantly longer in VAP group compared to non-VAP group. Although the mortality was also higher in neonates of VAP group, but it was not statistically significant.

Other studies have confirmed that VAP is associated with increased morbidity, a longer duration of mechanical ventilation, and a longer hospital stay, Fischer et al., [21] reported “an incidence of VAP of 9.6% in a neonatal and pediatric population after cardiac surgery and found a delay in extubation of 3.7 days attributable to VAP”. Similarly, Srinivasan et al., [22] Elward et al. [23] reported “an increased length of stay and a longer duration of ventilation in pediatric and neonatal VAP patients with a tendency towards increased mortality that did not reach statistical significance”.

Apistharnarak et al. [24] found “VAP to be an independent predictor of mortality in very low birth weight infants: moreover, VAP significantly increased the length of stay in NICU”. Tripathi et al.[25] reported “significantly higher length of stay in neonates with VAP. Although they reported higher mortality rates in VAP patients, it was statistically insignificant”.

“On the other hand, Xiao L., et al. reported no significant decreases in the incidence of respiratory infections, mortality or the rate of hospitalization in healthy children from vitamin

D supplementation” [26]. “Also, Yakoob M., et al. concluded, that there was no effect of vitamin D on the occurrence of pneumonia; nor any effect in children with pneumonia” [27].

Despite several systematic reviews and meta-analyses related to the effects of vitamin D on acute respiratory tract infection, vitamin D status is not routinely tested for neonates at risk of VAP.

Our study has some limitations, including that it was single center study and the relatively small sample size. Future studies are needed to evaluate proper vitamin D dosing regimens, understand the role of vitamin D in preventing VAP as well as how normalizing vitamin D level in neonates at risk of VAP can improve the clinical outcome.

### **Conclusion:**

25-hydroxy vitamin D deficiency is an important risk factor for VAP development in neonates, and low vitamin D levels is associated with significant longer duration of mechanical ventilation, post extubation O<sub>2</sub> support and hospital stay in neonates with VAP.

### **Ethical Approval and Consent :**

**Written informed consent was obtained from the parents of all newborns before enrollment to the study. The study was approved by Ethics Committee of Faculty of Medicine, Tanta University.**

### **References**

- [1] Cernada M, Aguar M, Brugada, et al. Ventilator-associated pneumonia in newborn infants diagnosed with an invasive bronchoalveolar lavage technique: a prospective observational study. *Pediatr Crit Care Med.* 2013;14:55–61.
- [2] Charles MP, Kali A, Easow JM, et al. Ventilator-associated pneumonia. *Australas Med J.* 2014;7(8):334-44.

- [3] Willson DF, Hoot M, Khemani R, et al. Pediatric ventilator-associated infections: the ventilator-associated infection study. *Pediatr Crit Care Med*. 2017;18:e24–34.
- [4] Cantey JB, Wozniak PS, Pruszynski JE, et al. Reducing unnecessary antibiotic use in the neonatal intensive care unit (SCOUT): a prospective interrupted time-series study. *Lancet Infect Dis*. 2016;16:1178–84.
- [5] Cantey JB, Wozniak PS, Sánchez PJ. Prospective surveillance of antibiotic use in the neonatal intensive care unit: results from the SCOUT study. *Pediatr Infect Dis J*. 2015;34:267–72.
- [6] Dell’Orto V, Raschetti R, Centorrino R, et al. Short- and long-term respiratory outcomes in neonates with ventilator-associated pneumonia. *Pediatr Pulmonol*. 2019;54(12):1982–8.
- [7] Ryan BA, Kovacs CS. Maternal and fetal vitamin D and their roles in mineral homeostasis and fetal bone development. *J Endocrinol Invest*. 2021;44(4):643-59.
- [8] El-Kassas GM, El Wakeel MA, Elabd MA, et al. Vitamin D Status in Neonatal Pulmonary Infections: Relationship to Inflammatory Indicators. *Open Access Maced J Med Sci*. 2019;7(23):3970-4.
- [9] Walker VP, Modlin RL. The vitamin D connection to pediatric infections and immune function. *Pediatr Res*. 2009;65(5):106-13
- [10] Sibaii H, El-Zayat SR, El-Shaheed AA, et al. The Hidden Function of Vitamin D. *Open Access Maced J Med Sci*. 2016;4(4):591-5.
- [11] Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control*. 2008;36, 309-32
- [12] Dogan P, Ozkan H, Koksall N, et al. Vitamin D deficiency and its effect on respiratory distress syndrome in premature infants: results from a prospective study in a tertiary care centre. *Afr Health Sci*. 2020;20(1):437-43
- [13] Ombelet S, Barbé B, Affolabi D, et al. Best Practices of Blood Cultures in Low- and Middle-Income Countries. *Front Med (Lausanne)*. 2019;6:131.
- [14] Shen L, Wang F, Shi J, et al. Microbiological analysis of endotracheal aspirate and endotracheal tube cultures in mechanically ventilated patients. *BMC Pulm Med*. 2019;19(1):162.

- [15] He CS, Gleeson M, Fraser WD. Measurement of circulating 25-hydroxy vitamin d using three commercial enzyme-linked immunosorbent assay kits with comparison to liquid chromatography: tandem mass spectrometry method. *ISRN Nutr.* 2013;2013:723139.
- [16] Clancy N, Onwuneme C, Carroll A, et al. Vitamin D and neonatal immune function. *J Matern Fetal Neonatal Med.* 2013;26(7):639-46.
- [17] Lezhenko G, Pashkova O, Kraynya H. The vitamin D deficiency as a marker of risk of pneumonia among children under 3 years. 2016; 3(1): 99-106.
- [18] Charan J, Goyal JP, Saxena D, et al. Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis. *J Pharmacol Pharmacother.* 2012;3(4):300-3.
- [19] Christensen N, Søndergaard J, Fisker N, et al. Infant Respiratory Tract Infections or Wheeze and Maternal Vitamin D in Pregnancy: A Systematic Review. *Pediatr Infect Dis J.* 2017;36(4):384-91.
- [20] Maretzke F. Role of vitamin D in preventing and treating selected extraskeletal diseases-an umbrella review. *Nutrients.* 2020;12(4):969
- [21] Fischer JE, Allen P, Fanconi S. Delay of extubation in neonates and children after cardiac surgery: impact of ventilator-associated pneumonia. *Intensive Care Med.* 2000;26(7):942-9.
- [22] Srinivasan R, Song Y, Wiener-Kronish J, Flori HR: Plasminogen activation inhibitor concentrations in bronchoalveolar lavage fluid distinguishes ventilator-associated pneumonia from colonization in mechanically ventilated pediatric patients. *Pediatr Crit Care Med* 2011;12:21-27.
- [23] Elward AM, Warren DK, Fraser VJ. Ventilator-associated pneumonia in pediatric intensive care unit patients: risk factors and outcomes. *Pediatrics.* 2002;109(5):758-64.
- [24] Apisarnthanarak A, Holzmann-Pazgal G, Hamvas A, et al. Ventilator-associated pneumonia in extremely preterm neonates in a neonatal intensive care unit: characteristics, risk factors, and outcomes. *Pediatrics.* 2003;112:1283-9.
- [25] Tripathi S, Malik G, Jain A, et al. Study of ventilator associated pneumonia in neonatal intensive care unit: characteristics, risk factors and outcome. *Internet Journal of Medical Update-EJOURNAL.* 2010;5(1).
- [26] Xiao L. Vitamin D supplementation for the prevention of childhood acute respiratory infections: a systematic review of randomised controlled trials. *Br. J. Nutr.* 2015;114(7):1026-34.

[27] Yakoob M.Y. Vitamin D supplementation for preventing infections in children under five years of age. Cochrane Database Syst. Rev. 2016;2016(11):CD008824.

UNDER PEER REVIEW