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Vitamin D Level in Ventilator Associated Pneumonia in the Neonatal Intensive Care Unit

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Abstract

Background: Neonatal ventilator-associated pneumonia (VAP) is recognized as a nosocomial lower airway infection in neonates with invasive mechanical ventilation who present beyond 48 hours. Besides the role of Vitamin D in calcium and phosphorus homeostasis, and bone metabolism, 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. The objective was to study role of vitamin D level as early predictors of VAP in the neonatal intensive care unit**. Methods:** This was a prospective cohort study that was conducted on 48 neonates who admitted to the NICU at the Paediatrics department, Zagazig University Hospital during the period of the study. They were divided into 2 groups (VAP group) and (non-VAP group) 24 cases in each group. Vitamin D assessment was done by ELISA. **Results:** 25 (OH) vitamin D was significantly lower in VAP group than non-VAP group. There was significant positive correlation between vitamin D and weight, Apgar score 1 m and 5 min, PaO2 while there was significant negative correlation with duration in NICU and duration in MV, WBCs, CRP and PaCO2. The cut off value of 29.8, vitamin D showed 92.3% sensitivity, 100% specificity, with p value <0.001as a predictor for occurrence of VAP development.the vitamin D level is lower in preterm than full term babies in the VAP group. **Conclusion:** Low serum vitamin D levels may be a risk factor for VAP development in intubated neonates especially preterm babies.

Key words: Vitamin D Level - Ventilator Associated Pneumonia – Neonatal Intensive Care Unit

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. Intubation and mechanical ventilation are known risk factors for the acquisition of nosocomial pneumonia. VAP is one of the most frequently diagnosed nosocomial infections and "second most common cause for antibiotic use in neonatal intensive care units (NICUs) after early onset sepsis (**1**).

Azab *et al*.(2) reported that the VAP rate has declined from 36.4 VAP episodes/1000 ventilation days to 23/1000 after VAP prevention bundle implementation in their NICU at Zagazig University. Others reported VAP incidence in different NICUs in Egypt to be 57.1% and 25.9/1000 ventilation days (**3**). Incidence rates reported in the literature from NICUs in Thailand, Brazil, China, and India were 70.3, 52, 48.8, and 37.2/1000 ventilation days, respectively (**4).** The situation is markedly different in the developed world as incidence rates in the USA and Germany ranged from 1.9 to 2.2/1000 ventilation days (**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. 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. Besides, many studies have identified an association of respiratory infectious diseases and inadequate serum vitamin D (**7**).

Certain risk factors increase the susceptibility for neonatal respiratory diseases as prematurity, meconium aspiration, caesarian section delivery, gestational diabetes, maternal chorioamnionitis, and prenatal ultrasonographic findings, such as oligohydramnios and structural lung disorders (**6**).

The objective was to study Role of Vitamin D Level as Early Predictors of Ventilator Associated Pneumonia in the Neonatal Intensive Care Unit.

Patients and Methods

This prospective cohort study was conducted on 48 Neonates admitted to the NICU at the Pediatrics department, Zagazig University Hospital during the period from April 2023 to April 2024. An informed written consent was obtained from the parents of patients. Every parents of patient received an explanation of the purpose of the study and every patient had a secret code number.

Inclusion criteria includes both sexes, neonates with gestational age $\geq$ 32 weeks and neonates ventilated for more than 48 hours. **Exclusion criteria exclude** neonates with gestational age $\leq$ 32 weeks, neonates ventilated for $\leq$ 48 hours and neonates with congenital pneumonia or multiple congenital anomalies.

Patients were divided into two groups: VAP group: included 24 neonates and Non VAP group **included** 24 neonates. **All neonates were subjected to a)** Complete history taking including personal data: age and gender, complaint, present, past and perinatal history, developmental and dietetic history, vaccination history and family history. b) Full clinical examination either general examination or local examination for all systems by inspection, palpation, percussion, and auscultation. C) Laboratory investigations: venous blood samples (3 cm) were taken from each infant participating in the study and were divided into two aliquots. The first aliquot, 1 ml of venous blood was used for determination of CBC and the second aliquot, 2ml of venous blood was left to clot, and then the serum was separated by centrifugation and stored at -20°C for detection of serum, LFT, KFT, CRP and vitamin D levels.

Serum 25-Hydroxy vitamin D level was measured by Enzyme-linked immune sorbent assay (ELISA) technique. Venous blood was collected from a sterile venipuncture and put in tubes labeled with the patient's name. Serum tubes were centrifuged after it allowed to clot at room temperature for separation of serum from whole blood. The serum was drowned and stored at 2:8 °C.

Other investigations were done such as blood culture, non-bronchoscopic bronchoalveolar lavage (NB-BAL) and endotracheal culture after 2 days of mechanical ventilation and when VAP was suspected. Chest radiography on admission and repeated as required and arterial blood gases ($PaCO_2$, PaO_2). Monitoring of the ventilator settings, including Peak inspiratory pressure, peak end expiratory pressure, respiratory rate, inspiratory time (Ti) and fraction of inspired oxygen (FIO_2).

Diagnosis of ventilator associated pneumonia

To diagnose ventilator associated pneumonia (VAP), the CPIS was used. The CPIS integrates a combination of clinical, radiologic, physiological, and microbiological criteria into a numerical value that represents VAP if it is over 6. To determine VAP severity, different parameters such as duration of mechanical ventilation, positive culture results, 14 and 28-day sepsis-associated mortality. Mortality was measured since admission in ICU. Length of ICU stay was measured since admission in ICU **(8)**.Radiological examination revealed new or worsening infiltrates on chest X-ray or CT thorax. Culture and sensitivity test was done to confirm the etiology whether bacterial or nonbacterial pneumonia.

Clinical diagnosis was done by at least one of the following: a) Fever more than 38°C with no other cause **.** b) Non-bronchoscopic bronchoalveolar lavage (NB-BAL). c) Leukopenia (< 4 000 WBC/mm^3) or leukocytosis (more than or equal 12 000 WBC/mm^3). d) New onset of purulent sputum or change in character of sputum (color, odor, quantity, consistency**.** e) Suggestive auscultation (rales or bronchial breath sounds), rhonchi, wheezing. f) Worsening gas exchange (e.g., O_2 desaturation or increased oxygen requirements or increased ventilation demand **(9)**.

Determination of vitamin D status:

VD status was determined by measuring serum 25-hydroxy vitamin (25(OH)D) concentration, which is the main circulating form of VD. VD sufficiency was defined as a serum 25 (OH) VD level of ≥30 ng/mL. VD insufficiency was defined as a serum 25 (OH) VD level of 20–29 ng/mL whereas VD deficiency was defined as a serum 25 (OH) VD level of <20 ng/ml. The 25(OH) VD concentration was measured using a commercial ELISA kit **(10).**

Ethical considerations

The study was approved by the Ethics Committee of Faculty of Medicine, Pediatrics department, Zagazig University Hospital. There are adequate provisions to maintain privacy of participants and confidentiality of the data are as follows: The parents of patients were given the option of not participating in the study if they did not want to. We put code number to each participate with the name and address kept in a special file. We hide the patients name when we use the research. We used the results of the study only in a scientific manner and not to use it in any other aims.

Statistical analysis

Data was collected, coded then entered as a spread sheet using Microsoft Excel 2016 for Windows, of the Microsoft Office bundle; 2016 of Microsoft Corporation, United States. Data was analyzed using IBM Statistical Package for Social Sciences software (SPSS), (IBM SPSS Statistics

for Windows, Version 26.0. Armonk, NY: IBM Corp). Continuous data was expressed as mean ± standard deviation, while categorical data as numbers and percentage. A statistical value <0.05 was considered as significant. Analytic statistics including correlation analysis (using Spearman/Pearson's method) and the ROC Curve (receiver operating characteristic).

Results:

Table (1): Demographic data among the studied groups.

		VAP group (n=24)	Non-VAP group (n=24)	P value
Gestational Age	Mean ± SD	35.95±2.13	35.58±1.99	0.53
	Range	33-39	33-39	
Gestational Age	Preterm (<37 weeks)	19 (79.2%)	18 (75%)	0.38
	Full-term (≥37 weeks)	5 (20.8%)	6 (25%)	
Maternal age	Mean ± SD	26±3.68	24.87±4.20	0.33
	Range	20-32	20-32	
Weight(kg)	Mean ± SD	2322.9167±140.5	2736.5417±273.6	0.004
	Range	2107-2581	2304-3197	
Sex	Male	18 (75%)	15 (62.5%)	0.35
	Female	6 (25%)	9 (37.5%)	
Mode of delivery	VD	10 (41.7%)	10 (41.7%)	0.615
	CS	14 (58.3%)	14 (58.3%)	

Chi square test (X^2), were used to analyze categorical variables. Student "t" test was used to analyze normally distributed variables among 2 independent groups.

There was no significant difference between both groups regarding to demographic data.

Table (2): 25(OH) vitamin D among the studied groups.

		VAP group (n=24)	Non-VAP group (n=24)	P value
25(OH) vitamin D (ng/ml)	Mean ± SD	22.82±5.75	37.98±4.76	0.001
	Range	13.7-30	30.2-45	

Student "t" test was used to analyze normally distributed variables among 2 independent groups.

25 (OH) vitamin D was significantly lower in VAP group than Non-VAP group.

Table (3): Outcome among the studied groups.

		VAP group (n=24)	Non-VAP group (n=24)	P value
Duration in NICU (days)	Mean ± SD	22.71±4.65	15.54±3.47	0.02
	Range	15-30	10-20	
Duration on MV (days)	Mean ± SD	11.21±2.08	7±1.71	0.15
	Range	8-14	4-10	
Apgar score 1 m	Mean ± SD	4.16±0.63	5.08±0.88	0.001
	Range	3-5	4-7	
Apgar score 5 m	Mean ± SD	5.83±0.82	7.25±0.79	0.001
	Range	5-7	6-8	
Mortality	Yes	5 (20.8%)	2 (8.3%)	0.21
	No	19 (79.2%)	22 (91.7%)	

NICU: The Neonatal Intensive Care Unit; MV: Mechanical Ventilation. Chi square test (X^2), were used to analyze categorical variables. Student "t" test was used to analyze normally distributed variables among 2 independent groups.

Duration of NICU was significantly higher in VAP group than Non-VAP group while Apgar score 1 minute and 5 minutes were significantly lower in VAP group than Non-VAP group. There was no significant difference between both groups regarding to duration on MV and mortality.

Table (4): 25(OH) vitamine Din VAP patients

		Preterm group (n=19)	Full-term group (n=5)	P value
25(OH) vitamin D (ng/ml)	Mean ± SD	19.34±4.37	24.81±1.89	0.041
	Range	13.7-25	20-30	

Student "t" test was used to analyze normally distributed variables among 2 independent groups.

25 (OH) vitamin D was significantly lower in VAP Preterm group than VAP Full-term group.

Table (5): ROC curve of vitamin D in prediction of VAP.

Cut-off	AUC	Sensitivity	Specificity	PPV	NPV	P value
29.8	0.89	92.3%	100%	100%	91.7	<0.001

AUC: Area Under the Curve. ROC curve was used to detect cutoff values with optimum sensitivity and specificity.

At a cut off 29.8, vitamin D showed 92.3% sensitivity, 100% specificity, with p value <0.001.

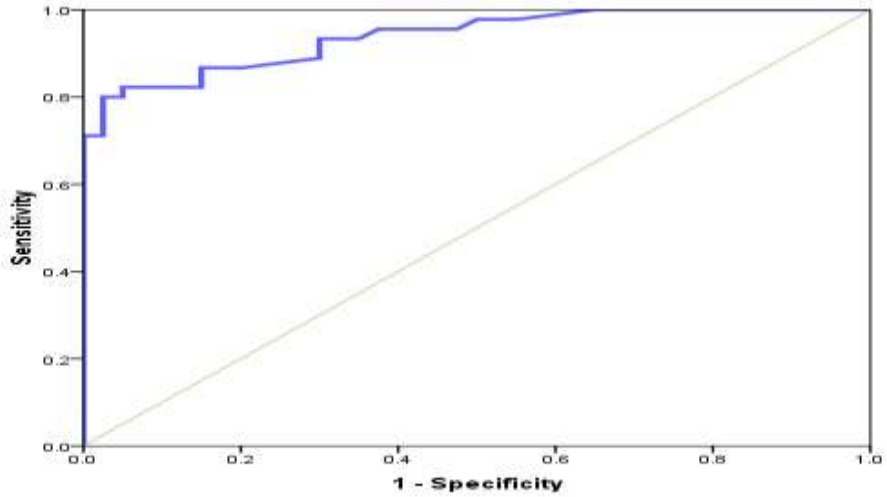

Figure (1): ROC curve of vitamin D in prediction of VAP.

Discussion

In our study, no significant differences were observed in gestational age (mean ± SD: 35.95 ± 2.13 weeks vs. 35.58 ± 1.99 weeks), maternal age (mean ± SD: 26 ± 3.68 years vs. 24.87 ± 4.20 years), sex distribution (75% males vs. 62.5% males), and mode of delivery (41.7% vaginal vs. 41.7% vaginal). However, the mean weight of neonates in the VAP group (2322.9167 ± 140.5 g) was significantly lower than that of the non-VAP group (2736.5417 ± 273.6 g), with a p-value of 0.004.
This slightly agrees with **Khattab et al. (11**) who reported that, the mean birth weight of the VAP group was significantly lower than that of the non-VAP group (P = 0.05). the mean gestational age of infants diagnosed with VAP was significantly lower than that of the non-VAP group.
In the current study we found that male predominance (75%; 62.5% respectively) in NICU population was noticeable among the studied groups.
This is in accordance with the study done by **Liu et al., (12**) who found significant male predominance in PICU population was noticeable. For almost 3 boys admitted there only one girl (male to female ratio = 3:1).
In our study, there was no significant difference between both groups regarding to indication of NICU admission and maternal risk factors.
In our study, Mucopurulent endotracheal tube secretion, auscultatory chest finding, radiological finding and progression of infiltration were significantly higher in VAP group than Non-VAP group while there was no significant difference regarding to fever.

This is in agreement with **Khattab et al. (11**) who reported that, hypothermia, presence of auscultatory chest findings, and mucopurulent ETT secretions characterized the VAP group.
Chest radiographs were diagnostic in all cases clinically diagnosed as VAP, which was in agreement with the results of **Erbay *et al.* (13).**
In our study, WBCs, leukocytosis and platelets were significantly higher in VAP group than Non-VAP group while there was no significant difference between both groups regarding to hemoglobin and leukopenia. In our study, CRP was significantly higher in VAP group than Non-VAP group while there was no significant difference between both groups regarding to serum albumin, blood culture and other laboratory findings.
Afify et al., (14) explained by the fact that CRP level can be affected by any infectious or inflammatory focus and not specific for VAP.
In harmony, **Khattab et al. (11**) who reported that, there were significant differences between VAP and non-VAP groups regarding total leukocyte count and CRP titer. Hypoalbuminemia, which is considered an indicator of poor nutritional status, was significantly encountered in the VAP group, which may be due to favored hepatic production of acute-phase proteins such as globulins, fibrinogen, and haptoglobin.
This study showed that, regarding outcome of the study groups death rate was (20.8%; 8.3% respectively). There was no significant difference between both groups regarding to duration on MV and mortality.
Khwannimit et al., (15) found the overall mortality rate was 44.5%.
This was lower **Rady, (16)** who found mortality rate was 33.1%. **Taori et al., (17**) found that the mortality rates was 17%.
This agreed with **Banupriya et al., (18)** who found that, there was no statistically significant difference in mortality between the two groups (p = 0.407).
While, **Tayel et al. (19**) reported that, mortality was significantly higher among VAP-diagnosed neonates (91.7%) compared with the non-VAP group (43.5%).
In our study, PaCO2 was significantly higher in VAP group than Non-VAP group while PaO2 was significantly lower in VAP group than Non-VAP group.
This agrees with **Tayel et al. (19**) who reported that, an increased attacks of hypoxia (PO_2<50), and hypercapnia (PCO_2>55) were significantly higher in the VAP group.
This was in agreement with the results that reported nearly the same observations (**4; 20**).
In our study, 25 (OH) vitamin D was significantly lower in VAP group than Non-VAP group.
This agrees with **Liu and Xu (21**) who aimed to investigate the correlation between the serum 25(OH)D_3 level in the cord blood of premature infants and the prognosis of NRDS. They indicated that the 25(OH)D_3 level in the serum of newborns with NRDS was statistically decreased than the control group, suggesting that the low 25(OH)D_3 of newborns may be related to NRDS. The level of serum 25(OH)D_3 of newborns could be used to predict NRDS taking 57.69 nmol/L (24ng/ml) as the cut-off value.
In our study, at a cut off 29.8, vitamin D showed 92.3% sensitivity, 100% specificity, with p value <0.001.
Ayad et al. (22) reported a cut off value of ≤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:

Low serum vitamin D levels may be a risk factor for VAP development in intubated neonates specially in preterm babies.

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