

## ASSESSMENT OF SERUM LEVEL OF VITAMIN D IN NEONATAL RESPIRATORY DISTRESS SYNDROME

By

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### ABSTRACT

**Background:** Previous studies have shown the relationship between lung development and vitamin D, but there have been few studies to assess vitamin D level in preterm babies have respiratory distress syndrome (RDS).

**Aim of the work:** The aim of this work is to determine the role of neonatal plasma vitamin D levels on occurrence of respiratory distress syndrome and correlated its level with the degree and the severity of the disease.

**Patients and methods:** This study included 30 preterm infants, recruited from neonatal intensive care unit of Toukh Central Hospital following informed consent from the parents during the period from July 2017 till February 2018. They are subdivided into two groups; one group with 30 preterm neonates diagnosed as having respiratory distress syndrome, the other group with 30 healthy preterm neonates. Full history taking, clinical examinations and investigations for all neonates are including serum 25-hydroxy vitamin D levels in both neonates.

**Results:** In 25 % of preterm infants, 25(OH) D levels are compatible with severe deficiency ( $\leq 10$  ng/mL), 38.3 % with moderate deficiency (10 – 20 ng/mL), and 26.7 % with mild deficiency (20 – 30 ng/mL). A normal 25(OH) D level is observed in none of the babies.

**Conclusion:** lower neonatal 25(OH) D levels are associated with neonatal respiratory distress syndrome.

**Keywords:** Respiratory distress syndrome, Premature, Vitamin D.

### INTRODUCTION

Respiratory Distress Syndrome (RDS) of the newborn is an acute lung disease caused by surfactant deficiency. It is seen primarily in neonates younger than 36 weeks

gestational age and weighing less than 2500 gm [1]. Vitamin D is a fat-soluble steroid hormone that contributes to the maintenance of normal calcium homeostasis and skeletal mineralization. Vitamin D

also has immunomodulatory effects on immune function [2]. Preterm infants are at increased risk for vitamin D deficiency also vitamin D status at birth is entirely dependent on maternal vitamin D status [3]. Respiratory distress syndrome (RDS) of the newborn is the most common cause of respiratory failure in preterm infants and results in significant morbidity and mortality. Its pathophysiology complex, but the absence of surfactant is one of the hallmark features of RDS [4]. Respiratory distress syndrome (RDS) also known as hyaline membrane disease (HMD), it occurs almost exclusively in premature infants. The incidence and severity of RDS are inversely related to the gestational age of the infant [5]. Vitamin D is a fat-soluble steroid hormone that contributes to the maintenance of normal calcium homeostasis and skeletal mineralization [6]. Vitamin D also has immunomodulatory effects on immune function [2]. Vitamin D is intimately involved in the growth and development of fetal lung including surfactant production [7]. Some studies have also revealed that, 25(OH) Vitamin D improves the maturation of type II pneumocytes and the synthesis of surfactant [8]. The studies are investigating the relationship

between the 25(OH) Vitamin D levels in babies and newborns with sepsis, bronchopulmonary dysplasia, atopic dermatitis, and asthma [9]. **Thomas et al.** have demonstrated that there is a strong correlation between the 25(OH) Vitamin D level of the mother and fetus [10]. **Wei et al.** have demonstrated that there is a correlation between 25 (OH) Vitamin D deficiency in the mother and preterm birth [11]. **Fernandez-Alonso and Dionis-Sanchez** have shown a correlation between lower 25 (OH) Vitamin D levels and the incidence of preeclampsia [12]. **Ataseven et al.** stated that vitamin D status is not correlated with gestational age [13].

### **AIM OF THE WORK**

The aim of this work is to determine the role of neonatal plasma vitamin D levels on occurrence of respiratory distress syndrome and it is correlated level with the degree and the severity of the disease.

### **PATIENT AND METHODS**

#### **Ethical consideration:**

1. Approval of the study protocol by Ethical Scientific Committee of AL\_Azhar University Assiut is obtained.

2. Informed consents, is taken from the parents before their enrollment in the study.
3. All the collected data of the studied case and results are confidential and the patient has the right to know them.
4. The patient and caregiver has the right to withdraw from the study at any time.
5. The authors declared that there is no potential conflict of interest with respect the research, authorship, and/or publication of this article.
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This case control prospective study is conducted on preterm neonates admitted to Neonatal Intensive Care Units (NICUs) of the Pediatric Department Toukh Central Hospital during the period from July 2017 till February 2018. This study included 60 preterm infants, they are subdivided into two groups; the 1<sup>st</sup> group included 30 preterm neonates less than 35 weeks diagnosed as having respiratory distress syndrome. The 2<sup>nd</sup> group included 30 healthy preterm neonates and exclude from the study full term neonates, infant with major congenital abnormalities or chromosomal

disorders, and maternal diseases affecting Vitamin D level (Gestational diabetes, parathyroid disease, calcium metabolism problems, and renal diseases).

All participants are subjected to the following maternal, prenatal, natal, postnatal history, general, systemic clinical examination, and investigations in the form of Serum 25-hydroxy vitamin D (25 (OH) Vitamin D) levels in neonates. 4ml of venous blood samples are obtained in the first day of life from babies in which 2ml of blood are added to the test tubes containing K-EDTA for analyzing the CBC and other two milliliters are added to red topped tubes without anti-coagulant. The red topped tubes are left for 20 minutes to coagulate, and then centrifuged for 20 minutes at 3000 rpm. The serum are separated and stored at -40°C until quantitative analysis for estimation of serum Vitamin D. All the previous analysis is performed by **EDITM 25-OH Vitamin D EIA Kit** with **REF (KT 815), LOT (J280)** and assay range from (12.6-74.6). Vitamin D deficiency is classified according to serum 25(OH) D levels as follows: < 10 ng/mL: Severe, 10 – 20 ng/mL: Moderate, 21 – 30 ng/mL: Mild vitamin D deficiency and > 30 ng/mL: Normal [11, 12].

### Statistical Analysis:

The collected data are analyzed using SPSS version 21.0-computer software (SPSS Inc, Chicago, ILL Company). Categorical data are presented as number and percentages using Chi square test (X<sup>2</sup>) and Fisher's exact test to analyze them. Continuous variables are tested for normality using Shapiro-Wilks test, Quantitative data are expressed as mean ± standard deviation and range if normally distributed, using Student "t" test to compare means of 2 parametric variables or expressed as median and IQR

using Man Whitney U test (MWU) for two non-parametric variables. Krauskal Wallis test (KWT) is used to compare 3 non parametric variables. Spearman's correlation coefficient (rho) is used to test correlations between non-parametric variables. ROC curve is used to detect the cutoff value of vitamin D level with optimum sensitivity and specificity in the prediction occurrence of RDS and its severity. The accepted level of significance in this work is stated at 0.05 (P <0.05 is considered significant).

## RESULTS

**Table (1): Comparison of the studied groups regarding gestational age and birth weight**

Variable	Patients (N=30)			Controls (N=30)			St. "t" Test	P Volume
	Mean	±SD	Range	Mean	±SD	Range		
Gestational age (w)	32.5	1.47	30-35	33.0	1.09	32-35	1.48	0.14 (NS)
Weight (kg)	1.76	0.26	1.3-2.3	1.78	0.32	1.3-2.5	0.24	0.81 (NS)

Table (1) shows that, non-statistical significant decrease in the patients group than control

group regarding to gestational age and birth weight.

**Table (2): Comparison the studied groups regarding Vitamin D level**

Variable	Patients (N=30)			Controls (N=30)			St."t"	P
	Mean	±SD	Range	Mean	±SD	Range		
<b>Vit D level (infant)</b>	9.94	4.54	2.8-19	24.95	6.36	10-36		0.001 (HS)

Table (2), highly statistical significant decreases in the patients than the control group

regarding to infant Vitamin D. While,

**Table (3): Comparison the studied groups regarding degree of infant Vitamin D deficiency**

Infant vit D		group		Total
		Patients	Controls	
Normal	Count	0	6	6
	% within group	.0%	20.0%	10.0%
Mild Vit D deficiency 21-30 ng/mL	Count	0	16	16
	% within group	.0%	53.3%	26.7%
Moderate vit D deficiency 10-20 ng/mL	Count	15	8	23
	% within group	50.0%	26.7%	38.3%
Severe < 10 ng/mL	Count	15	0	15
	% within group	50.0%	.0%	25.0%

Table (3) shows the statistical significant decrease between the patients than the controls group is regarding to the percentage of

degree of infant Vitamin D deficiency (mild, moderate and sever deficiency).

**Table (4): Correlation between the infant Vitamin D level and the studied continuous variables**

With	Vit D			
	Patients		Controls	
	Rho	P	rho	P
Maternal age	-0.289	0.12	0.006	0.97
Gestational age	0.594	0.001 (HS)	0.022	0.91
Birth weight	-0.150	0.42	0.071	0.71
APGAR 1	0.455	0.012 (S)	0.430	0.018 (S)
APGAR 5	0.278	0.13	0.234	0.21
RR	-0.443	0.014 (S)	0.151	0.42
Down score	-0.695	<0.001 (HS)	0.117	0.53
Grade of RD	-0.650	<0.001 (HS)	----	-----
Hb%	-0.470	0.009 (S)	-0.355	0.07
TLC	-0.144	0.45	0.028	0.88
PLTs	0.247	0.18	-0.329	0.076

Table (4) shows that, a significant negative correlation between infant Vitamin D level and down score and Hb %, a highly significant negative correlation between infant Vitamin D and grades of RDS among x-ray, a significant

positive correlation between infant Vitamin D and gestational age, Apgar score at 1st minute. Non-significant correlation between infant Vitamin D level and birth weight, TLC, and platelet count.

**Table (5): Validity and predictivity of cut-off point of Vitamin D level in prediction of occurrence of respiratory distress syndrome in preterm babies**

Variable	Sens%	Spec%	PPV%	NPV%	Accuracy%	AUC	95%CI AUC
Infant Vit D ≤ 17.2 ng/ml	96.7%	93.3%	93.5%	96.5%	95%	0.97	0.93-1.0

Table (5) shows the performance of infant Vitamin D in Prediction of preterm with respiratory distress syndrome. At a cut off value ≤ 17.2ng/L,

sensitivity of infant Vitamin D is 96.7% with 93.3% specificity, PPV 93.5, NPV 96.5 and accuracy: 95%.

## DISCUSSION

This study included 60 preterm infants (35 males and 25 females) with mean of GA of  $33.3 \pm 1.3$  weeks. They are subdivided into (Group 1 : 30 preterm infants with respiratory distress syndrome, Group 2 : 30 preterm infants without respiratory distress syndrome served as control group). In the current study, patients have non-statistical significant difference regarding gestational age and birth weight. The same results are reported by **Loughrey et al.**, they found no significant difference in infants of RDS when compared to infants without RDS, in gestational age more than 30 weeks [14]. Moreover, **Wang et al.** indicate that there is highly significantly association between birth weight, gestational age, and RDS incidence [15]. They found that, RDS incidence increased with decreased birth weight and gestational age. Our study are showed that , there is a highly statistical significant decrease in the patients than the control group regarding to the infant Vitamin D and statistical significant decrease in the patients than the controls group regarding percentage of degree of infant Vitamin D deficiency.

In our case control prospective study, there is a high prevalence of a 25 OH D level  $\leq 30$  nmol/L among preterm infants, It also shows that, 25.0 % of preterm being severe, 38.3 % being moderate, and 26.7 % is mild 25 (OH) D deficiency. RDS is more frequent in babies with severe vitamin D deficiency (50.0 % of babies with severe and 50.0 % of babies with moderate deficiency have RDS). This result is similar with the study done by **Ataseven et al.**, ninety-seven (63%) of preterm is severe, 50 (34%) is moderate, and 5 (3%) is mild vitamin D deficiency [13]. There is no baby in the study with a normal 25 (OH) Vitamin D level. Five babies in the mildly deficient group are also included in the moderately deficient group. Two groups are designed: There are 97 babies with 25 (OH) Vitamin D level  $< 10$  ng/ml (group 1) and 57 babies with 25 (OH) Vitamin D level 10-30 ng/ml (group 2). Twenty-seven patients (28%) are diagnosed as RDS in the first group, while eight (14%) patients have RDS in the second group. RDS is more frequent in babies with severe 25 (OH) Vitamin D deficiencies. The risk of RDS is reduced to 3.34 times in babies with higher vitamin D levels. We indicate that, a significant negative correlation between infant

Vitamin D level and (Down score and Hb %).

A highly significant negative correlation between infant Vitamin D and grades of RDS among x-ray, a significant positive correlation between infant Vitamin D and (gestational age, Apgar score at 5th minute and non-significant correlation between infant Vitamin D level and (birth wt, TLC and platelet count).

Average 25 (OH) Vitamin D levels of girls are similar with the boys. In our study, we try to find the performance of infant Vitamin D in Prediction of preterm with respiratory distress syndrome. At a cut-off value  $\leq 17.2$  ng/L, sensitivity of infant Vitamin D is 96.7% with 93.3% specificity, PPV 93.5, NPV 96.5 and accuracy: 95%. **Ataseven et al.** investigated whether 25 (OH) Vitamin D deficiencies is a risk factor for RDS [13]. None of the preterm infants have normal vitamin D level, which underlined the burden of Vitamin D deficiency in pregnant women and their offspring. RDS is more common in severely vitamin D-deficient preterm. Determination of vitamin D status of the mothers and appropriate supplementation might be a valuable strategy to reduce RDS, in addition to antenatal steroids. Besides, since

vitamin D is a regulatory factor in many organs during fetal development.

### **CONCLUSION**

Lower neonatal 25 OH D levels are associated with neonatal respiratory distress syndrome and also 25 (OH) Vitamin D might be a promising agent in addition to antenatal steroids for the prevention of RDS.

Premature newborns are at great risk for vitamin D deficiency. A higher incidence of RDS in the group of infants with lower 25 (OH) Vitamin D levels presents the importance of monitoring 25 (OH) Vitamin D intake and levels during pregnancy in order to prevent RDS.

### **RECOMMENDATIONS**

Appropriate vitamin D supplementation to the pregnant women to prevent neonatal RDS in their off springs

We suggest the need for a multicenter randomized interventional trial to assess the effect of optimized vitamin D status on preterm infants' outcomes, especially respiratory outcomes.

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# تقييم نسبة فيتامين د في الدم في الأطفال حديثي الولادة المصابين بمتلازمة ضيق التنفس

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## الملخص العربي:

متلازمة ضيق التنفس تحدث في الأطفال حديثي الولادة (الأطفال المبتسرين) علي وجه الاخص ويسمي ايضا مرض الغشاء الشفاف، حيث يتناسب معدل حدوث المرض وشدته تناسباً عكسياً مع العمر الرحمي للجنين. يعد فيتامين دمن الستيرويد هرمون التي تذوب بالدهون ويعمل علي تنظيم معدل الكالسيوم في الجسم والعظم وايضاً له دور تحسن نمو الخلايا الحويصليه من النوع الثاني المبطنه للرئه وايضاً تصنيع الفاعل (السيرفاكتانت).

## الهدف من الدراسة:

ان الهدف من هذا العمل هو دراسة دور فيتامين د في حدوث متلازمة ضيق التنفس وكذلك درجة وشدة حدوثه بنسبة فيتامين د في الاطفال المبتسرين.

## الحالات وطرق البحث:

اشتملت هذه الدراسة علي 60 طفل من الاطفال المبتسرين (35 ذكور و25 إناث) مع متوسط عمر جنيني  $(1.3 \pm 33.3)$  أسابيع من حديثي الولادة في وحدة العناية المركزة في مستشفى طوخ

المركزي، وتم الحصول علي الموقفه علي برتوكول الرسالة من قبل اللجنة الاخلاقيه بجامعة الازهر\_اسيوط وكذلك الحصول علي الموافقه المستلمه من الوالدين قبل بدء الدراسة.

**المجموعة 1:** الأطفال المبتسرين الذين يعانون من متلازمة ضيق التنفس.

**المجموعة 2:** الأطفال المبتسرين الغير مصابين بمتلازمة ضيق التنفس اكلينيكيًا او عن طريق الاشعة (مواليد اصحاء).

وقد خضع كل المرضى للتالى : أخذ تاريخ مرضى دقيق (مثل العمر الرحمى والتاريخ المرضى للام وطريقة الولادة إذا كانت طبيعية أو قيصرية ،والفحص الاكلينيكي (الفحص العام الشامل وفحص الجهاز التنفسى وفحص الأجهزة الأخرى) وتم عمل الفحوصات الاتية:صورة دم كامله والبروتين سى التفاعلي وغازات الدم بالشريان وقياس 25 اكسيد فيتامين د في الاطفال وكذلك عمل اشعه تشخيصيه علي الصدر.

## نتائج البحث:

تشير نتائج الدراسة الحالية الاتي:

توجد اختلافات ذات دلالة احصائية كبيره بين المجموعتين بالنسبة لنسبة فيتامين د في الاطفال.

كما يوجد فرق كبير بين المجموعتين بالنسبه لدرجات نقص فيتامين د (بسيطه او متوسطه او شديده).

توجد ايضا علاقه ايجابيه بين نسبة فيتامين د في الاطفال والعمر الرحمي ومقياس ابجار.

وقد اظهر تحليل ROC للبيانات ان القيمه الفاصله لفيتامين د في الطفل وتنبأ حدوث متلازمه ضيق التنفس هو اقل من او يساوي 17.2 نانو جرام (حساسيته لحدوث متلازمه ضيق التنفس هو 96.7% وخصوصيته 93.3).

### الاستنتاجات:

يمكننا ان نستنتج مما سبق ان نقص نسبه فيتامين د عند المواليد يقترن بحدوث متلازمه ضيق التنفس في هؤلاء الاطفال.

ونستخلص من رساله ان متلازمة ضيق التنفس من أكثر الأمراض أهمية داخل وحدات العناية المركزة لحديثي الولادة وان الأطفال المبتسرين والذين يعانون من متلازمة ضيق التنفس عادة ما يعانون من قلة في العمر الرحمي والوزن لذلك نوصي بزيادة العناية والمتابعة في فترة الحمل لتقليل نسبة الأطفال المبتسرين.