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Association of vitamin D receptor gene FokI and TaqI polymorphisms and risk of RDS.

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Abstract

Background: Vitamin D and its receptor (VDR) have important roles in perinatal lung development. The objective of this study was to investigate the possible association between VDR FokI and TaqI polymorphism and development of respiratory distress syndrome (RDS) in preterm infants.

Method: A total of 173 premature infants <34 week: 82 with RDS and 91 without RDS were enrolled. Genotyping of VDR polymorphisms were assayed by real-time PCR. Serum 25-hydroxyvitamin D (25-OHD) levels were measured by ELISA in blood samples that were obtained at the time of admission to the neonatal intensive care unit.

Results: Gestational age (GA) was significantly lower in RDS group compared to the controls. In univariate analysis, VDR TaqI CT and CC genotypes were associated with the increased risk of RDS (OR = 3.264 , $P = 0.001$, 95% CI = 1.597-6.672 and OR = 5.222, $P < 0.001$, 95% CI = 2.165-12.597, respectively); while VDR FokI showed no association with RDS. 25-OHD levels in RDS group were significantly lower compared with those in without RDS group (P=0.002). Serum 25-OHD levels were not significantly different among the different FokI and TaqI genotypes.

Conclusions: This is the first report of association of VDR polymorphism with RDS development in preterm neonates. Current study suggests that VDR TaqI polymorphism may be involved in predisposition to RDS in premature neonates. Further studies are needed to assess the contribution of vitamin D and VDR signaling to the pathogenesis RDS

Keywords: preterm infants; RDS; vitamin D receptor (VDR) gene; single-nucleotide polymorphism; vitamin D

Introduction

Respiratory distress syndrome (RDS) is the most common cause of respiratory distress in preterm infants and causes significant morbidity and mortality. The deficiency in surfactant production and structural immaturity of the lung constitue the primary etiologies of RDS [1]. Despite surfactant replacement therapy, antenatal maternal steroid administration and optimum respiratory care, RDS continues to be a common problem for preterm infants. Although the incidence of RDS is inversely related to gestational age (GA), prematurity alone does not determine the risk of developing the disorder [2]. RDS is thought to be a multifactorial disease and some studies suggest that genetic factors may contribute in the pathogenesis [2,3].

Vitamin D, primary hormone for bone metabolism and calcium hemostasis, is suggested to play a role in the embryogenesis and cellular growth and differentiation, including lung development and regulation of lung maturation in the fetus [4-7]. Vitamin D receptors (VDR), acting as a transcription factor, mediate vitamin D effects including proliferation, differentiation, and complex regulations and widely distributed in many tissues [8]. Animal and laboratory studies demonstrated VDR expression on fetal lung alveolar type-II cells and positive effects of vitamin D on alveolar type-II cells growth, surfactant synthesis, and alveolarization [9,10].

Several epidemiological studies have described an association between some VDR gene variants with susceptibility to childhood respiratory infections, wheezing and asthma in children [11-13]. However, only limited data are available for the role of vitamin D on lung diseases early in life. Taking the knowledge of a potential role of vitamin D in lung development and maturation into account, we proposed VDR gene polymorphisms may be involved in the pathogenesis of RDS.

The numbers of single nucleotide polymorphisms (SNPs) are identified in the VDR gene, however among the most examined are SNPs located in exon 2 (FokI, rs2228570), and exon9 (TaqI, rs731236), named by the restriction enzymes used for their determination. The FokI polymorphism creates an alternative start codon in exon 2 which leads to production of a longer and less active form of protein (14). TaqI located in last exon, may influence VDR's RNA stability and translation efficiency and consequently the transcription of the target gene (14).

The aims of our study are to (i) compare serum 25(OH)D levels between patient and control groups; (ii) investigate whether VDR polymorphisms could be a risk factor for preterm newborn with RDS; and (iii) determine whether different genotypes of the VDR polymorphisms influence serum 25(OH)D levels and clinical course in preterm newborns with RDS.

Materials and methods

Study population

This prospective study was carried out in the level III Neonatal Intensive Care Unit of Dr. Sami Ulus Maternity and Children Research and Training Hospital, Ankara, Turkey, from September 2012 to September 2013. One hundred and seventy-three preterm infants with gestational age less than 34 weeks were enrolled in this study. Detailed history was taken for all neonates including birth weight, gestational age, gender, mode of delivery, maternal history of antenatal steroid use, premature rupture of membranes (PROM), diabetes mellitus and preeclampsia. Also, Apgar score was recorded. Participitants were classified into two groups: preterm newborns with RDS

(# = 82) and those without RDS (# =93). Participitants were classified into two groups: preterm newborns with RDS (# = 82) and those without RDS (# =93).
RDS was defined as the presence of clinical signs of respiratory distress (tachypnea, retractions, flaring, grunting, or cyanosis), with a requirement for supplemental oxygen in conjunction with a characteristic chest radiograph. The chest radiographic features of RDS include a low lung volume and the classic diffuse reticulogranular ground glass appearance with air bronchograms. Other causes of respiratory distress were distinguished from RDS by their clinical features, radiographic features, and course[15]
Also, infants of gestational age (GA) of less than 28 weeks, 28–31 weeks, and 6 d and 32–34 weeks were regarded as extremely preterm, very preterm and moderate to late preterm, respectively [2]. Exclusion criteria were presence of congenital anomalies, genetic disorders, intrauterine infections, inherited metabolic disorders. All infants were born Turkish families living in the Central Anatolia region of Turkey.

All newborns with respiratory distress were followed on nasal continuous positive airway pressure (CPAP) as an initial respiratory support. Surfactant was given as both as an early rescue and prophylactic treatment. SIMV (synchronized intermittent mandatory ventilation) providing optimal tidal volume was the primary mode of ventilation in infants who could not be extubated immediately.

This study was approved by the Local Ethics Committee and written informed consent was obtained from parents of infants This study was conducted in accordance with the Declaration of Helsinki.

Laboratory evaluation for serum 25-OHD levels

Blood samples of the enrolled infants were obtained at the time of admission to the neonatal intensive care unit during routine blood sampling. For measurement of 25-OHD levels, 2mL of total blood were obtained in a non-anti-coagulant tube and stored at s-20oC until the time of analysis. The 25(OH)D levels were measured using the APPLIED 3200 Biosystem (DPC Cirrus Inc., Diagnostic Products Corporation Los Angeles, CA) device following 2 h of incubation at room temperature with the BioSource 25OH-Vit.D3-Ria-CT kits (BioSource Europe S.A. Rue de I'Industrie, 8, B–1400 Nivelles, Belgium). We considered vitamin D deficiency if 25(OH) D value < 20 ng/ml and insufficiency if 25(OH) D value between 20 and 30 ng/ml. Vitamin D deficiency was further classified as severe deficiency <10 ng/ml and moderate deficiency10–20 ng/ml [16].

Genotyping

After taking informed consent, whole peripheral blood samples were collected in tubes containing Ethylene Di-amine Tetra-acetic Acid (EDTA) and stored at −20 °C until processing. The genomic DNA was extracted from the frozen EDTA-blood samples by using the EZ1 DNA Blood 200 µl Kit (Qiagen) according to the manufacturer's instructions. Single nucleotide polymorphisms (SNPs) were selected based on the functional relevance and minor allele frequency (> 0.05) using genotype data obtained from Caucasian individuals in the 1000 genome project. In our study, we examined 2 SNPs on the VDR gene: FokI (rs2228570) (tagging rs10735810) and TaqI (rs731236). Genotyping was performed at the Dıskapı Yıldırım Beyazıt Traning and Research Hospital (Ankara, Turkey) using prevalidated Hybprobe probe SNP genotyping system. For all reactions, Roche Light Cycler Nano (Roche Diagnostics, Mannheim, Germany) instrument and LightCycler® FastStart DNA Master HybProbe (Roche Diagnostics, Mannheim Germany) were used. The data were analyzed using Roche LightCycler

NanoSoftware 1.0. PCR mix contained 10.4 µl PCR grade water (H2O), 1.0 µl Reagent mix, 2.0 µl FastStart DNA master Hybprobe, 1.6 µl MgCI2 (25 mM) and 5.0 µl (50 ng) DNA. The mixture was distributed to the microplate wells, 20 µl for each reaction. The plate was placed into the cycler, after centrifugation. The amplification conditions consisted of one denaturation/activation (for Hot-Start Taq) cycle of 10 min at 95°C and 45 cycles of three-temperature amplification. Each cycle was 95°C for 10 sec, 60°C for 10 sec, and 72°C for 15 sec with a single fluorescent acquisition step at the 60°C hold. This step was followed by a melting curve analysis of 95°C for 30 sec, 40°C for 120 sec, and a slow ramp (1°C/sec) to 75°C with continuous fluorescent acquisition.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) for windows 20.0 software (SPSS Inc, Chicago, Illinois, USA) was used to evaluate the data. These data are summarized as mean ± SD and percentages.Genotype and allele frequencies were compared using Chi-square and Fisher's exact test. Deviations from Hardy–Weinberg equilibrium were evaluated by the Chi-square test. Dominant genetic model was applied for genotype distribution analysis. The Student t-test was used to compare normally distributed variables in independent groups, and the Mann–Whitney U-test was used to compare nonparametric variables. Comparisons between the two groups were made using the Chi-square and Fisher's exact tests for categorical variables. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters. Bonferroni correction for multiple testing was used for polymorphism studies and a p value of <0.017 (0.05/3) was considered statistically significant. Gestational age, birth weight and gender were adjusted in the binary logistic regression model for the association of VDR polymorphisms with RDS. The odds ratio and 95% confidence intervals were calculated. Statistical significance was considered at $p < 0.05$.

Results

A total of 173 preterm infants were enrolled in this study. Of these 173 infants, 82 (47.3%) were diagnosed as RDS and 91 (52.6%) were controls. Gestational age, birth weight, Apgar scores at 5th minute, need for mechanical ventilation and duration of ventilation were found significantly different between RDS and control groups. Gender, mode of delivery and antenatal medical history including steroid use, premature rupture of membrane, preeclampsia and maternal diabetes were not significantly different between the two groups. There was no difference between groups with respect to maternal age, maternal educational status. However, mothers of the RDS group were less likely to use vitamin D regularly, which was statistically significant (P = 0.004). The demographic and clinical characteristics of the RDS and control group are summarized in Table 1. The RDS rate in extremely preterm, very preterm, and moderate to late preterm neonates were 81.8%, 52.6%, and 23.4 %, respectively (p=0.0001). Mortality rate was higher in the RDS group than the controls (15.8 versus 3.3%, respectively) (p=0.007) (Table 1).

The mean serum 25(OH)D level was significantly lower in RDS group than control group (13.78±5.50 vs 16.70±6.59 ng/ml; p=0.002).Results of the two-way ANCOVA on the confounding impact of gestational age showed that the significant differences in terms of 25(OH)D continued (F=4.409, p = 0.03) (Table 1).Only three infants in RDS group had normal 25(OH)D levels, 10 (12.2%) had insufficiency and 69 (84.2%) had severe or moderate deficiency; meanwhile 7 (7.7%) infants in control group had normal 25(OH)D levels,30 (33%) had insufficiency and 54 (60.4%) had severe or moderate deficiency. There was no statistically significant correlation between

serum 25(OH)D level and gestational age (r=0.067; p=0.382) and birth weight (r= -0.006; p=0.936).

Distribution of FokI (rs2228570) and TaqI (rs731236) and genotypes and alleles among studied subjects were shown in Table 2. The genotype frequencies of FokI and TaqI in patient and control groups were compatible with Hardy–Weinberg expectations (FokI p= 0.14; x2 = 0.278; TaqI p= 0.14, x2=2.77). In univariate analyses, significant association was detected between the TaqI (rs731236) polymorphism and RDS ($p < 0.001$) and after using the Bonferroni correction for multiple testing, the TaqI polymorphism remained significantly associated with RDS (p corrected = 0.017). CT genotype was associated with a 3.26 times increased risk of RDS (p = 0.001, 95% CI = 1.597-6.672) and CC genotype was associated with 5.22 times increased risk of RDS ($p< 0.001$, 95% CI = 2.165-12.597). Likewise, the presence of C allele was associated with 2.60 times increased risk of RDS. ($p<0.001$, 95%CI=1.678-4.044). However, there was no significant variation in the genotype and allele frequency distribution between patients and controls for FokI (rs2228570) (p = 0.498 and p=0.287 respectively).

Binary logistic regression analysis revealed that the negative effect of the TaqI polymorphism was preserved when adjusting for gestational age, birth weight and male gender, with the carriers of the having a 3.6-fold higher probability for RDS (P = 0.001, OR = 3.464, 95% CI = 1.655–7.251). However, FokI polymorphism did not have any effect in the same model (P = 0.445, OR = 0.772, 95% CI = 0.396–1.501).

To explore the impact of VDR FokI and TaqI polymorphisms on serum 25(OH)D levels in patients with RDS, we compared 25-OHD serum levels of participants carrying different genotypes of VDR polymorphisms (Table 4). Based on genotypic distribution, we found no significant association between serum 25-OHD levels and FokI and TaqI polymorphisms (p=0.331 and p=0.367, respectively). The genotypes of FokI and Taq I polymorphism did not markedly influence the mechanical ventilation duration, mortality, and antenatal steroid use (Table 4).

Discussion

To the best of our knowledge, this is the first study that evaluates possible associations between both 25(OH)D serum levels and VDR polymorphisms and RDS in preterm neonatesWe found lower serum levels of 25(OH)D in preterm infants with RDS compared to controls. Moreover, VDR TaqI rs73123 was significantly associated with RDS in preterm after adjusting gestatinal age, birth weight and gender.FokI, rs2228570 demonstrated no relationship with RDS in same model. On the other hand, there was no significant connection between FokI and TaqI genotypes and 25(OH)D serum levels.

Vitamin D is suggested to play a role in the morphogenesis of lung and surfactant production in fetus [18]. It was demonstrated that, 1,25(OH)D increased surfactant synthesis by increasing intracellular phosphatidylcholine and phosphatidylglycerol and increased surfactant secretion in type two pneumocytes [19].

Preterm infants are at risk of vitamin D deficiency because the transplacental transfer of 25(OH)D mainly occurs during the last trimester [20]. In our study, vitamin D deficiency was detected in 71% of all included preterm infants; 84.2% in RDS group and 60.4% in control group.

A growing body of evidence denotes that there is a strong relationship between 25(OH)D and RDS.; on the other hand, there are controversial reports on serum 25(OH)D levels of patients with RDS. Hegazy et al. [21] reported significant correlation between vitamin D level and respiratory distress syndrome in 65 preterm neonates under

the gestational age of 34 weeks. Fettah et al. [22] found significantly increased risk for respiratory distress in infants with vitamin D levels lower than 15 ng/mL that low 25(OH)D level was associated with development of RDS. However, Onwuneme et al. [23] reported no significant between vitamin D level and respiratory distress in preterm infants. In present study, neonates in RDS group had significantly lower mean serum 25(OH)D level than the control group ((13.78±5.50 vs 16.70±6.59 ng/ml; $p<0.001$).

Data from human and animal studies suggest that VDR are found in fetal lung alveolar type-II cells primarily during the last period of gestation when ATII cells differentiate, surfactant biosynthesis and secretion begin (24).It has been reported that the metabolically active form of vitamin D, 1,25(OH)D upregulates VDR in ATII cells during the pseudoglandular and saccular stages of lung development where proximal and distal airways are formed, respectively (25).

TaqI polymorphism is located at the 30 UTR region of the VDR gene, which is involved in the regulation of mRNA stability and protein translation, and therefore the altered VDR level can influence the vitamin D signaling efficiency [14]. TaqI polymorphism although is nonfunctional, it is considered to be linked with other functional polymorphisms and participates in a more complex gene network enhancing or inhibiting the expression of VDR target genes [14]. Thus, TaqI polymorphism that appears to be associated with RDS in our study may result in a decreased activity of VDR. Disturbance of VDR function may be important for the developing lungs, especially for surfactant synthesis and secretion. There was no significant variation in the genotype and allele frequency distribution between RDS patients and controls for FokI.

In present study, although patients with TaqI CC gentotype had higher serum 25(OH)D levels we did not discover significant relationship between either TaqI or FokI with serum 25-OHD level. Morrison et al. [26] suggested that TaqI CC genotype have been associated with decreased VDR function and elevated levels of 1,25(OH)2D3. Tayel SI et al. [27] reported that vitamin D deficiency and VDR FokI polymorphism were risk factor for neonatal sepsis. Also, FokI was found to be related with serum 25(OH)D level. Although not significant, TaqI CC and CT genotypes and C allele frequencies were common in neonatal sepsis cases while TT was common in healthy control in that study. Haldar et al. [28] reported that TaqI TT genotype carried a significant protection associated with acute lower respiratory infections and no connection between VDR genotypes and serum 25(OH)D level. Several studies suggested that TaqI polymorphism was associated with asthma in children [12,29]. Koroglu et al. [30] examined the role of the VDR polymorhisms in BPD risk among 109 preterm neonates but did not find a significant relation. Funke et al. [31] investigated the influence of Taq 1 polymorphism of VDR on bone disease of preterm infants. The distribution of Taq1 polymorphism of VDR gene was not significantly different in that study.

Smaller sample size was one of our limitations. Further genome-wide studies on larger population of preterm neonates are needed. Lack of data on prenatal maternal 25(OH)D levels was another limitation in our study. We have studied only two SNPs in the VDR gene, other VDR polymorphisms (i.e., BsmI, and ApaI,) should be genotyped concurrently with evaluation of vitamin D status in infants with RDS to validate these findings on different ethnic populations. Interventional trials of vitamin D supplementation are also needed.

In conclusion, we found possible relationship of VDR TaqI polymorhism with risk of RDS development in Turkish preterm infants. We also found serum 25(OH)D levels to be significantly lower in patients with RDS. These findings suggest that 25(OH)D is involved in the pathophysiology of RDS. Our results should be considered as preliminary and require confirmation in future studies.

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Declaration of interest

The authors report no conflicts of interest.

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Table 1. Demographic and clinical characteristics of the study groups.
Table 2. Distribution of VDR FokI and TaqI polymorhisms in RDS and control group
Table 3. Effect of VDR FokI and TaqI Polymorphisms on mechanical ventilation duration, mortality, antenatal steroid use and serum 25-OHD levels in RDS group

Table 1. Demographic and clinical characteristics of the study groups

Table 1: Demographic and clinical characteristics of the study groups

	RDS (n=82)	Control (n=91)	*p*
Gestational age (wk) Mean ± SD	29.06±2.7	31.12±1.6	<0.0001
Birth weight (g) Mean ± SD	1298.40±468.1	1583.63±408.5	<0.0001
Gender, n (%)			
Female	37 (45.1)	48 (52.7)	0.369
Male	45 (54.9)	43 (47.3)	
C/S, n (%)	51 (62.2)	61 (67)	0.506
Apgar score (5th min) Mean ± SD	6.16±1.4	7.26±0.8	<0.0001
Mechanical ventilation, n (%)	66 (80.5)	11 (12.1)	<0.0001
Duration of ventilation (d) Mean ± SD	4.21±4.16	1.13±2.23	<0.0001
Mortality, n (%)	13 (15.8)	3 (3.3)	0.007
25-OHD ng/ml Mean ± SD	13.78±5.50	16.70±6.59	0.002[a]
Age of mother (y) Mean ± SD	29.3±5.2	28.9±5.2	0.662
Vitamin D usage of mother, n (%)			0.004
None	19 (23.2)	10 (11)	
Irregular	48 (58.5)	45 (49.5)	
Regular	15 (18.3)	36 (39.6)	
Maternal education, n (%)			0.367
Unschooled	29 (35.4)	23 (25.3)	
Less than high school	37 (45.1)	47 (51.6)	
High school or more	16 (19.5)	21 (23.1)	
Antenatal steroid, n (%)	61 (74.3)	72 (79.1)	0.476
Gestational diabetes, n (%)	5 (6.1)	4 (4.4)	0.615
Preeclampsia, n (%)	13 (15.9)	10 (11)	0.347
Premature rupture of membranes, n (%)	36 (43.9)	37 (40.7)	0.666

[a] The significance was continued after ANCOVA (F= 4.409, P = 0.03).

Table 2: Distribution of VDR FokI and TaqI polymorhisms in RDS and control group

Table 2: Distribution of VDR FokI and TaqI polymorhisms in RDS and control group

SNP ID	Genotype/Allel	RDS n (%)	Control n (%)	*p*	OR (%95 CI)	*p*
FokI (rs2228570)	CC	47 (57.3)	44 (48.3)			
	CT	27 (32.9)	36 (39.6)	0.498	0.702 (0.368-1.340)	0.283
	TT	8 (9.8)	11 (12.1)		0.681 (0.251-1.849)	0.449
	CT+TT	35 (42.7)	47 (51.6)		0.697 (0.382-1.271)	0.238
	C	121 (73.7)	124 (68.1)			
	T	43 (26.3)	58 (31.9)		1.316 (0.825-2.100)	0.287
TaqI (rs731236)	TT	18 (21.9)	47 (51.6)			
	CT	40 (48.8)	32 (35.2)	<0.001**	3.264 (1.597-6.672)	0.001**
	CC	24 (29.3)	12 (13.2)		5.222 (2.165-12.597)	<0.001**
	CT+CC	64 (78)	44 (48.4)		3.798 (1.953-7.387)	<0.001**
	T	76 (46.3)	126 (69.2)			
	C	88 (53.7)	56 (30.8)		2.605 (1.678-4.044)	<0.001**

Table 3: Effect of VDR FokI and TaqI Polymorphisms on mechanical ventilation duration, mortality, antenatal steroid use and serum 25-OHD levels in RDS group.

	FokI (rs2228570)				Taq I (rs731236)			
	CC	CT	TT	*p*	TT	CT	CC	*p*
Mechanical ventilation, n (%)	36 (76.6)	23 (85.2)	7 (87.5)	0.582	14 (77.8)	31 (77.5)	21 (87.5)	0.588
Duration of ventilation (d) Mean ± SD	4.83±4.80	3.67±2.90	2.38±3.20	0.220	3.40±3.39	4.53±4.10	4.29±4.84	0.632
Antenatal steroid use, n (%)	36 (76.6)	19 (70.4)	5 (62.5)	0.710	13 (72.2)	31 (77.5)	16 (66.7)	0.635
Mortality, n (%)	8 (17)	4 (14.8)	1 (12.5)	0.934	2 (11.1)	7 (17.5)	4 (16.7)	0.820
Serum 25-OHD Mean ± SD (ng/ml)	13.62±4.67	14.74±7.04	11.50±3.58	0.331	13.17±5.82	13.25±5.17	15.13±5.78	0.367

Table 1. Demographic and clinical characteristics of the study groups

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	RDS (n=82)	Control (n=91)	*p*
Gestational age (wk) Mean ± SD	29.06±2.7	31.12±1.6	<0.0001
Birth weight (g) Mean ± SD	1298.40±468.1	1583.63±408.5	<0.0001
Gender, n (%)			0.369
Female	37 (45.1)	48 (52.7)	
Male	45 (54.9)	43 (47.3)	
C/S, n (%)	51 (62.2)	61 (67)	0.506
Apgar score (5th min) Mean ± SD	6.16±1.4	7.26±0.8	<0.0001
Mechanical ventilation, n (%)	66 (80.5)	11 (12.1)	<0.0001
Duration of ventilation (d) Mean ± SD	4.21±4.16	1.13±2.23	<0.0001
Mortality, n (%)	13 (15.8)	3 (3.3)	0.007
25-OHD ng/ml Mean ± SD	13.78±5.50	16.70±6.59	0.002[a]
Age of mother (y) Mean ± SD	29.3±5.2	28.9±5.2	0.662
Vitamin D usage of mother, n (%)	19 (23.2)	10 (11)	0.004
None	48 (58.5)	45 (49.5)	
Irregular	15 (18.3)	36 (39.6)	
Regular			
Maternal education, n (%)			0.367
Unschooled	29 (35.4)	23 (25.3)	
Less than high school	37 (45.1)	47 (51.6)	
High school or more	16 (19.5)	21 (23.1)	
Antenatal steroid, n (%)	61 (74.3)	72 (79.1)	0.476
Gestational diabetes, n (%)	5 (6.1)	4 (4.4)	0.615
Preeclampsia, n (%)	13 (15.9)	10 (11)	0.347
Premature rupture of membranes, n (%)	36 (43.9)	37 (40.7)	0.666

aThe significance was continued after ANCOVA (F= 4.409, P = 0.03).

Table 2: Distribution of VDR FokI and TaqI polymorhisms in RDS and control group

SNP ID	Genotype/Allel	RDS n (%)	Control n (%)	*p*	OR (%95 CI)	*p*
FokI (rs2228570)	CC	47 (57.3)	44 (48.3)			
	CT	27 (32.9)	36 (39.6)	0.498	0.702 (0.368-1.340)	0.283
	TT	8 (9.8)	11 (12.1)		0.681 (0.251-1.849)	0.449
	CT+TT	35 (42.7)	47 (51.6)		0.697 (0.382-1.271)	0.238
	C	121 (73.7)	124 (68.1)			
	T	43 (26.3)	58 (31.9)		1.316 (0.825-2.100)	0.287
TaqI (rs731236)	TT	18 (21.9)	47 (51.6)			
	CT	40 (48.8)	32 (35.2)	<0.001**	3.264 (1.597-6.672)	0.001**
	CC	24 (29.3)	12 (13.2)		5.222 (2.165-12.597)	<0.001**
	CT+CC	64 (78)	44 (48.4)		3.798 (1.953-7.387)	<0.001**
	T	76 (46.3)	126 (69.2)			
	C	88 (53.7)	56 (30.8)		2.605 (1.678-4.044)	<0.001**

Table 2: Distribution of VDR FokI and TaqI polymorhisms in RDS and control group

Pearson chi-square test **p<0.01

Table 4: Effect of VDR FokI and TaqI Polymorphisms on mechanical ventilation duration, mortality, antenatal steroid use and serum 25-OHD levels in RDS group.

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