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Research Article
Impact of Maternal Vitamin D Receptor (VDR) Gene Polymorphisms on Spontaneous Preterm Birth (Egyptian Case-Control Study)

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Abstract

Background and Objective: Many polymorphisms had been mapped in vitamin D receptor (VDR) gene on chromosome 12, they had variable location which influences its functional prospect. The present study aimed to evaluate the possible association between *VDR* gene *Fok*I SNP (C>T, rs2228570) polymorphism and Spontaneous Preterm Birth (SPTB) in the Egyptian pregnant population. **Materials and Methods:** A total of 160 pregnant women, divided into two groups: 80 preterm (case group) and 80 control groups, were investigated for VDR gene *Fok*I polymorphism by predesigned Taq-Man SNPs genotyping assay. **Results:** The Odds Ratio (OR) for the preterm birth risk was significantly higher with the mutant homozygous (TT) genotype ($p = 0.01$). Vitamin D level is significantly different among the three *VDR Fok*I SNP genotypes in both groups. The lowest vitamin D level was found in variant homozygous TT genotype (19.6 and 25.5 ng mL^{-1} in preterm and control groups, respectively). The C allele was associated with low preterm birth risk [OR: 0.43 (0.20-0.91)] and the T allele was associated with high preterm labor risk [OR: 2.0 (1.04-3.79)]. **Conclusion:** The *VDR Fok*I polymorphism is associated with an increased risk of Spontaneous Preterm Birth (SPTB) in Egyptian pregnant women. It may play a possible role in SPTB etiology. The current study provides data on the consideration of *VDR Fok*I polymorphism as a potential biomarker for SPTB.

Key words: *Fok*I, real-time PCR, Taq man assay, SNPs, VDR, spontaneous preterm birth

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Preterm birth (PTB) is a primary etiological agent of neonatal mortality accounting for 70% of all perinatal deaths and results in long-term morbidity in the respiratory and nervous system in survivors[1]. The exact etiology of Spontaneous Preterm Birth (SPTB) is not completely known, with several reports linking it with subclinical, intraamniotic, intrauterine or even extrauterine infections. The organism's possible origin is variable, whether from urinary, reproductive, or ascending from the cervix. About 80-90% of vitamin D is obtained through thermal reaction converting 7-dehydrocholesterol in the epidermis of the skin into vitamin D, while the other 10-20% of vitamin D is obtained through the diet[2,3].

The relation between vitamin D and immune response has been suggested besides its principal function in the regulation of calcium balance and bone formation[4]. Experimental studies suggested a possible relationship between vitamin D status and placental antibacterial activity. Three postulated mechanisms of the relationship between vitamin D levels and PTB are known; first, suboptimal vitamin D level reduces Toll-like receptor-mediated production of antimicrobial cathelicidin from macrophages, which decrease bacterial infection[5]. Second, vitamin D reduces cytokine expression such as interleukin-6, interleukin-13, lipopolysaccharide (LPS), Granulocyte-macrophage colony-stimulating factor 2 (GMCSF-2) and tumor necrosis factor (TNF)α in myometrial, smooth muscle and uterine smooth muscle (UtSM) cells[6]. Eventually, Contractile-associated Proteins (CAPS) expression in UtSM cells is attenuated by calcitriol, creating another mechanism of its effect in a spontaneous preterm birth reduction. The action of vitamin D after combination with Vitamin D Receptor (VDR) is one of the steroid hormone receptor family results that regulates transcription of many genes[7]. Many polymorphisms had been mapped in *VDR* genomic site on chromosome 12, which had variable location that influences its functional prospect.

Single Nucleotide Polymorphism (SNP) is the most prevalent genetic variation in the human genome[8-10]; nonsynonymous mutation within exons may have an impact on developing many diseases, also influence many diseases, mainly complex conditions like diabetes and cancer, because it might affect protein expression, conformation and function and mRNA splicing and stability[11-13]. *VDR Fok*I SNP resulted from T/C substitution at exon 2, which leads to alteration of the first transcription site of the *VDR*, resulting in a protein with 424 amino acids, which is three amino acids shorter than the original VDR. The relationship between *VDR* gene *Fok*I SNP and SPTP had been evaluated scarcely in the literature with contradictory results[14,15]. This condition had not been analyzed in Egyptian pregnant women. Thus, the present study aimed to assess the possible association between *VDR* gene *Fok*I SNP (C>T, rs2228570) and SPTP in the Egyptian pregnant population.

MATERIALS AND METHODS

Experimental section

Study design: A case-control study was performed on pregnant women attending the outpatient obstetrics and gynecology clinic at Tertiary Hospital, Zagazig University, from February, 2019 to May, 2020. Assuming that Vitamin D levels in the preterm and control groups were 20.8±11.8 and 25.8±10.7 ng mL^{-1}, respectively, at confidence level 95% and power 80%, the total sample size was 160 pregnant women (80 in every group) calculated by Open Epi version 2.3.1[16]. The enrolled subjects were divided into two groups. The first group enclosed women with SPTB second group included women who have full-term birth exclusion criteria included multifetal pregnancy, fetal anomalies or growth restriction, chronic inflammatory conditions and antepartum hemorrhage. Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University, Maternity Hospital approved the study and written consent was acquired from all participants.

Samples and DNA extraction: Peripheral blood (10 mL) was collected at the time of delivery from each participant by peripheral venipuncture in two tubes; first tube, containing a clot-separating gel used for biochemical analysis. Samples tubes were centrifuged at 1500×g for 10 min; then the plasma was aliquoted into microtubes and frozen at -20°C for further analysis. Vitamin D levels were determined by electrochemiluminescence using an Elecsys® Vitamin D Total II kit (Roche Diagnostics GmbH, Mannheim, Germany). The Endocrine Society defined the following cutoff levels to describe vitamin D status; deficient <20 ng mL^{-1}, insufficient 20-29 ng mL^{-1} and sufficient ≥30 ng mL^{-1}[17]; these values were adopted in the current study.

The second tube was containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant agent for genetic studies and DNA extraction; collected tubes were stored at -20°C until further research. DNA was extracted using DNA-mini-kit (Genotek, Ottawa, Canada) following the manufacturer's instructions. Extracted DNA was diluted with free nuclease water to 5-20 ng μL^{-1}.

Polymorphism selection and genotyping of VDR *Fok*I (rs2228570) using real-time PCR: The genotyping for the VDR *Fok*I SNP (rs2228570) was conducted using a predesigned Taq-Man SNPs genotyping assay that includes a mixture of predesigned unlabeled PCR-primers and the Taq-Man Minor Groove Binding group (MGB) probes and FAM and VIC as dye-labeled (Applied Biosystem-USA). Taq-Man PCR Master Mix Consists of DNA polymerase, dNTPs and Optimal mix ingredients utilize general thermal conditions. The real-time PCR was conducted in 96 well-plates in an ABI 7000 instrument (Applied Biosystems, California, USA) with a final reaction volume of 15 µL that consist of 1.0 µL genomic DNA, 8.0 µL Taq-Man Genotyping Master Mix (2×), 1.0 µL Taq-Man probes (assay mix-20×) and filled up to 15 µL with Milli-Q water (DNase and RNase free). The PCR conditions were performed as follows: pre-denaturing at 95°C for 5 min, then 40 cycles of 95°C for 15 sec and 60°C for 60 sec.

Statistical analysis: The collected data was entered to and analyzed by computer using SPSS Statistics package (SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.) and Epi Info 7 version 7.2.0.1[18]. Shapiro-Wilk test was used to determine the distribution characteristics of variables and variance homogeneity. Quantitative data were presented as a mean±standard deviation. Qualitative data were presented as frequencies and proportions. Pearson Chi-square test and Chi-square test for linear trend (χ^2) were used to analyzing qualitative independent variables and to estimate the Hardy-Weinberg Equilibrium (HWE). The HWE test was performed using Michael H. 'Court's online calculator as described by Chahil *et al.*[19]. 'Student's t-test was used to analyze quantitative independent variables. Odds Ratio (OR) and 95% confidence interval (95% CI) were used to assess the strength of association between VDR gene polymorphism and PTB. One-way analysis of variance (ANOVA) and post hoc (LSD) was used to test differences in vitamin D levels between and within VDR *Fok*I genotypes. Binary logistic regression was done to describe the relationship between PTB (as dependent variable) and significantly related variables to assess PTB predictors. All the tests are two-tailed and a p-value of $\leq$0.05 was taken as significant[20].

RESULTS

A total of 160 pregnant women, divided into two groups: 80 preterm (case group) and 80 control group from the same ethnic group, were investigated for *VDR* gene *Fok*I polymorphism. The sociodemographic characteristics of the participated subjects were summarized in Table 1. The previous miscarriage, history of prior preterm birth, gestational diabetes mellitus (GDM), cesarean section and Chorioamnionitis were significantly higher in the preterm birth group in comparison with the control group. In contrast, gestational age and birth weight were significantly lower in the preterm birth group in comparison with the control group ($p \leq 0.001$). There was a statistically significant difference

Table 1: Characteristics of the studied groups

Variables	Preterm group (n = 80)	Control group (n = 80)	Test of sig.	p-value
Age (years)[x]	28.2±6.3	26.5±5.2	t 1.8	0.1
Pre-gestational BMI (kg m^{-2})[x]	28.0±4.7	27.2±5.1	t 1.0	0.3
History of previous preterm birth	33 (41.3%)	16 (20.0%)	χ^2 8.5	0.004*
History of previous miscarriage	18 (22.5%)	8 (10.0%)	χ^2 4.6	0.03*
Intake of vitamin D supplementation	6 (7.5%)	10 (12.5%)	χ^2 1.1	0.3
Gestational diabetes mellitus	27 (33.8%)	8 (10.0%)	χ^2 13.2	0.001**
Urinary tract infection	15 (18.8%)	10 (12.5%)	χ^2 1.2	0.3
Pregnancy induced hypertension	16 (20.0%)	11 (13.8%)	χ^2 1.1	0.3
Mode of delivery				
Vaginal delivery	14 (17.5%)	42 (52.5%)	χ^2 21.5	0.001**
Cesarean section	66 (82.5%)	38 (47.5%)		
Gestational age (weeks)[x]	26.0±2.4	34.3±3.5	t 17.4	0.001**
Neonatal Birth weight (gm)[x]	2033.0±296.3	2934.4.3±322.4	t 18.4	0.001**
Chorioamnionitis				
Yes	24 (30.0%)	5 (6.3%)	χ^2 15.2	0.001**
No	56 (70.0%)	75 (93.7%)		
25-OHD (ng mL^{-1})[x]	24.4±7.2	28.6±6.6	t 3.9	·0.001**
Sufficient (>32)	12 (15.0%)	26 (32.5%)		
Insufficient (20-32)	43 (53.8%)	45 (56.3%)	χ^2 12.7	0.002*
Deficient (·20)	25 (31.3%)	9 (11.3%)		

[x]Data presented as Mean±SD, BMI: Body mass index, 25-OHD: 25-hydroxyvitamin D, *Statistically significant, **Highly statistically significant

Table 2: Hardy-Weinberg equilibrium for *VDR* gene *Fok*I SNP genotypes in the studied groups

Genotypes of *VDR* gene *Fok*I	Preterm group		Control group	
	Observed	Expected	Observed	Expected
CC	25	20	38	36
CT	30	40	29	35
TT	25	20	13	10
Hardy-Weinberg equilibrium	0.03*		0.1	

*Not consistent with HWE. VDR: Vitamin D receptor, CC: Allele CC, CT: Allele CT, TT: Allele TT

Table 3: Genotyping and allele frequency of *VDR* gene *Fok*I SNP genotypes in the studied groups

Genotypes of *VDR* gene polymorphism	Preterm group (n = 80)	Control group (n = 80)	OR (95% CI)	p-value
CC	25 (31.3%)	38 (47.5%)	Reference	1
CT	30 (37.4%)	29 (36.3%)	1.57 (0.77-3.22)	0.2
TT	25 (31.3%)	13 (16.2%)	2.92 (1.26-6.76)	0.01*
C allele (CC+CT)	55 (68.8%)	67 (83.8%)	0.43 (0.20-0.91)	0.02*
Non-C allele (TT)	25 (31.2%)	13 (16.2%)		
T allele (TT+CT)	55 (68.8%)	42 (52.5%)	2.0 (1.04-3.79)	0.03*
Non-T allele (CC)	25 (31.2%)	38 (47.5%)		

*Statistically significant, VDR: Vitamin D receptor, OR: Odds ratio, CC: Allele CC, CT: Allele CT, TT: Allele TT

Table 4: Association between serum vitamin D_2 level and *VDR* gene *Fok*I SNP genotypes in the studied groups

*VDR Fok*I SNP genotypes	25-OHD (ng mL)[a]	
	Preterm group	Control group
CC	29.6±5.1	32.2±5.5
CT	24.0±6.4	25.3±5.8
TT	19.6±6.4	25.5±6
F	16.1	14.2
p-value	<0.001**	0.001**
LSD	0.01*[b]	0.92[b]
Post hoc	0.00**[c]	0.01*[c]
	0.00**[d]	0.00**[d]

[a]Data presented as Mean±SD, [b] CC versus CT, [c] CC versus TT and [d] CT versus TT. 25-OHD: 25-hydroxy vitamin D, LSD: Least significant difference, * Statistically significant, ** Highly statistically significant

Table 5: Binary logistic regression for predictors of preterm birth

Variables	Exp (B)	95% CI	p-value
History of previous preterm birth	4.8	1.92-8.83	0.01*
Previous miscarriage	0.99	0.99-1.0	0.9
Gestational diabetes mellitus	5.6	1.12-9.80	0.009*
Chorioamnionitis	2.0	1.20-12.32	0.05*
Serum 25-OHD	0.93	0.88-0.98	0.008*
***VDR Fok*I SNP genotypes**			
CC (reference)	-	-	-
CT	0.98	0.96-2.60	0.7
TT	2.3	1.12-9.67	0.01*

*Statistically significant, 25-OHD: 25-hydroxyvitamin D, CC: Allele CC, CT: Allele CT, TT: Allele TT

between preterm and control groups in vitamin D as the preterm group had a lower vitamin D than the control group (Table 1). The percentage of women with sufficient, insufficient and deficient vitamin D levels was 15.0, 53.8 and 31.3%, respectively, in the preterm group compared to 32.5, 56.3 and 11.3%, respectively in the control group. Vitamin D deficiency is more common in the preterm group, while sufficiency is more prevalent in the control group.

*VDR Fok*I genotypes were obtained from the TaqMan predesigned primer assay. According to Hardy-weinberg Equilibrium (HWE), there are three genotypes: Homozygote reference (CC), heterozygote (CT) and homozygote variant (TT) given in Table 2. In contrast, to the control group, the *VDR Fok*I genotypic frequency in the preterm group was not consistent with HWE (<0.05).

As illustrated in Table 3, Homozygote reference (CC) was found in 31.3 and 47.5% in cases and controls, respectively. Homozygote variant (TT) was found in 31.3 and 16.2% in cases and controls, respectively. Taken the CC genotype as a reference, the OR for the preterm birth risk was significantly higher with the mutant homozygous (TT) genotype (p = 0.01). Heterozygote (CT) was found in 37.4 and 36.3% in cases and controls, respectively. There was no statistically significant difference regarding the distribution of heterozygous genotype in both studied groups. In alleles analysis, the C allele was associated with low preterm birth risk [OR: 0.43 (0.20-0.91)] and the T allele was associated with high preterm labor risk [OR: 2.0 (1.04-3.79)].

Vitamin D level is significantly different among the three *VDR Fok*I SNP genotypes in both groups in Table 4. The lowest vitamin D level was found in variant homozygous TT genotype in preterm and control groups. Post hoc analysis illustrated the difference within genotypes. In the preterm group, there were significant differences between the three genotypes. In the control group, there were substantial differences in TT genotype versus other genotypes. On the other hand, there was no statistically significant difference between CC and CT. The regression model for preterm birth predictors revealed that previous preterm birth; GDM, Chorioamnionitis, serum vitamin D level and TT genotype were statistically significant showed in Table 5.

DISCUSSION

Spontaneous Preterm Birth (SPTB) could be a multifactorial condition that incorporates genetic, hormonal and ecological factors that influence its activities. Crider *et al.*[21] reported that the gene-environment interactions better describe the risk of PTB and if this is the case, polymorphisms within critical genes could be used to describe the risk. The current results of the case-control study showed that among risk factors for triggering SPTB, women who had a history of previous preterm birth history of prior miscarriage and gestational diabetes mellitus are more likely to be delivered preterm. Moreover, the mode of delivery in the study group is a caesarian section in 82.5% of patients, which is significantly higher when compared to the control group ($p \leq 0.001$). It is also noticed that Chorioamnionitis, despite being a rare event in the control group it is more common in preterm occurring in 24 of 80 cases (30%). The difference between both groups regarding Chorioamnionitis is highly statistically significant ($p<0.001$). Placental transfer of vitamin D from mothers to fetus constitutes the only source of fetal vitamin D, so the maternal and fetal levels are closely linked[3,22]. Sufficient vitamin D level decreases colonization by bacterial vaginosis, which may partially explain the vitamin D effect in reducing preterm delivery rates[23].The current results indicated that serum levels of 25-hydroxyvitamin D (25-OHD) were significantly lower in the preterm birth group vs. the control group, which may suggest that vitamin D deficiency is a risk factor for preterm birth; these results agree with[1,24-27]. A meta-analyses with a considerable sample size were conducted by Qin *et al.*[26] included 11393 patients from 11 observational studies found that pregnant women with 25-OHD<20 ng mL^{-1} had a risk of premature birth increased about 1.3 times (OR, 1.29, 95%CI, 1.16-1.45). Amegah *et al.*[1] investigated 18 studies [Four Randomized Controlled Trials (RCTs) and 14 observational studies] compromising 18471 patients and concluded that women with vitamin D deficient had an 86% elevated Relative Risk (RR) for preterm birth and those with vitamin D insufficiency (20-29 ng mL^{-1}) had 24% higher preterm birth risk (RR = 1.24) 18 observational trials and six RCTs were included in a study by Zhou *et al.*[27] who demonstrated that maternal vitamin deficiency led to increased risk of preterm birth (RR = 1.25, 95% CI:1.13-1.38).

The impact of vitamin D on the innate immune system is the most contributing mechanism in its effect in preventing preterm birth. Identification of VDR on macrophages and dendritic cells indicate valuable action of 25-OHD in activating these cells to produce antimicrobial peptide[28-30]. These antimicrobial peptides are essential in fighting against perinatal infections, which are etiological factors that are seen in association with preterm birth. The impact of vitamin D as an immuno-modulating substance organizing endometrial cellular cytokine production obviously plays an essential role in preterm birth etiology[31]. Placental expression of *VDR* and 1-alpha hydroxylase is observed throughout pregnancy, indicating a valuable local function of vitamin D in fetoplacental development cellular differentiation and maternofetal signaling[32]. Moreover, vitamin D decreases cytokine expression such as interleukin-6, LPS, GMCSF-2 and (TNF)α in UtSM cells. On the other hand, the current results contradict with Pérez-López *et al.*[33] and Roth *et al.*[34]. Most of the genomic studies of SPTB focused on inflammation and immunity-related genes like TNFα, IL2, IL4 and IL1β because of the well-defined role of immune modulation in SPTB process. The demonstration of decreased *VDR* gene mRNA expression in the placental tissue from the preterm birth cases compared to normal term birth placenta[35]. This role is played by for mentioned genes and by vitamin D through *VDR* and activation of *VDR* in target cells. The only SNP which affects VDR protein structure is located on 5'' end of *VDR* gene *Fok*I polymorphism.

Being located in a coding sequence, this polymorphism is functional; it is mapped at the beginning of the start codon and it involves T/C substitution at exon 2. On starting translation from this new site, a longer protein product is composed of 424 amino acids, which is three amino acids shorter than the wild type; this shorter protein has a low transcriptional activity, which subsequently leads to lower activation in the target cells. For the first time, the present results elucidate an association between *VDR Fok*I SNP and preterm birth risk. Taken the CC genotype as a reference, the OR for the preterm birth risk was significantly higher with the mutant homozygous (TT) genotype ($p = 0.01$). The C allele was associated with low preterm birth risk [OR: 0.43 (0.20-0.91)] and the T allele was associated with high preterm labor risk [OR: 2.0 (1.04-3.79)]. Vitamin D level is significantly different among the three *VDR Fok*I SNP genotypes in both groups. The lowest vitamin D level was found in variant homozygous TT genotype in preterm and control groups. Results of the present study consistent with El-Beshbishy *et al.*[36], who found an association between *Fok*I polymorphism and prematurity risk in Saudian population; also Manzon *et al.*[37] investigated four *VDR* related SNPs (*Bsm*I, *Taq*I, *Fok*I and *Apa*I)in 33 Israeli population with PTP and 98 blood samples from full-term, uncomplicated singleton births and reported that the frequency of *Fok*I C allele was significantly higher in women that had PTP. Also, the current results are in accordance with Javorski *et al.*[38] who stated that *Fok*I T allele was related to

preterm birth risk. Another investigation by Barchitta *et al.*[39] was conducted for evaluating 17 mothers and their preterm neonates and 187 full-term control mothers with their neonates. They established that there was a relation between *Fok*I polymorphic genotype and increased risk of preterm birth. In contrast with the current results, Dutra *et al.*[40] showed that wild homozygous *Fok*I CC genotype was associated with preterm delivery. In comparing genotype frequencies of 100 preterm women with 99 full-term controls, Baczyńska-Strzecha, Kalinka[41] showed that differences were found. Still, the combinations of *Apa*I/AA, *Bsm*I/bb, *Taq*I/TT and *Apa*I/aa, *Bsm*I/BB, *Taq*I/tt genotypes were significantly higher in PTB group. In contrast, the *Bsm*II/Bb, *Taq*I/Tt, *Apa*I/AA and *Bsm*I/BB, *Taq*I/tt, *Apa*I/Aa, combinations decrease the risk of PTB. On the other hand, Rosenfeld *et al.*[42] compared 146 mothers and their preterm neonates with 229 other mothers and their full-term neonates and found that the CC homozygous genotype of the *Apa* I-SNP was related to PTB risk. The current results regarding the association between *Fok* I TT genotype and Chorioamnionitis indicate possible rule of *Fok* I SNP, the etiopathogenesis of infection and inflammation, which are mechanisms involved in preterm labor. The present study has a number of limitations as small sample size that generalizes the results difficult unless repeated on a larger cohort, lack of nutritional and anthropometric observations on studied women, prevents proper investigation of the relationship of the nutrition status and prematurity risk, vitamin D level and SPTB rate, despite the limitations mentioned above, the current study is unique in determining the relationship between vitamin D value, *Fok*I polymorphism and preterm birth risk. The uniform genetic makeup of the studied women nullifies any ethnic effect during *Fok*I-SNP analysis. Despite extensive research, lake of understanding the proper relationship between genetic contributions and SPTB is evident.

CONCLUSION

Mutant homozygous genotype (TT) of *VDR* gene *Fok*I SNP is associated with the lowest vitamin D level and significantly increased risk of SPTB in Egyptian pregnant women. It may play a possible role in SPTB etiology. The current results provide data on the consideration of *Fok*I as a potential biomarker for SPTB, but further large-scale research in other populations still needed to underline this finding. Current study provides preliminary data concerning the elaborate genetic makeup associated with an elevated risk of SPTB, which may help clinicians invent ways to prevent or delay SPTB. Further genetic linkage assessment studies to determine the direct effect of the *Fok*I polymorphism regarding SPTB risk are needed. Identification of possible responsible mechanisms and genetic factors linked with SPTB could find targets for better diagnostic and therapeutic interventions.

SIGNIFICANCE STATEMENT

This study discovered that *VDR Fok*I polymorphism is associated with an increased risk of Spontaneous Preterm Birth (SPTB) in Egyptian pregnant women that can be beneficial as a biomarker. This study will help the researchers to uncover the direct effect of the *Fok*I polymorphism and SPTB risk. Thus, it may explain a possible role of *VDR Fok*I polymorphism in SPTB etiology.

REFERENCES

1. Amegah, A.K., M.K. Klevor and C.L. Wagner, 2017. Maternal vitamin D insufficiency and risk of adverse pregnancy and birth outcomes: A systematic review and meta-analysis of longitudinal studies. PLoS ONE, Vol. 12. 10.1371/journal.pone.0173605.
2. Momentti, A.C., D. Estadella and L.P. Pisani, 2018. Role of vitamin D in pregnancy and toll-like receptor pathway. Steroids, 137: 22-29.
3. Hossein-Nezhad, A. and M.F. Holick, 2013. Vitamin D for health: A global perspective. Mayo Clinic Proc., 88: 720-755.
4. Martin Hewison 2011. Antibacterial effects of vitamin D. Nat. Rev. Endocrinol., 7: 337-345.
5. Liu, N.Q. and M. Hewison, 2012. Vitamin D, the placenta and pregnancy. Arch. Biochem. Biophys., 523: 37-47.
6. Sehringer, B., W.R. Scha¨fer, B. Wetzka, W.R. Deppert, R. Brunner-Spahr, E. Benedek and H.P.Zahradnik, 2000. Formation of proinflammatory cytokines in human term myometrium is stimulated by lipopolysaccharide but not by corticotropin-releasing hormone. J. Clin. Endocrinol., Metabolism, 85: 4859-4865.
7. Whitfield, G.K., J.C. Hsieh, S. Nakajima, P.N. MacDonald and P.D. Thompson *et al.*, 1995. A highly conserved region in the hormone-binding domain of the human vitamin D receptor contains residues vital for heterodimerization with retinoid X receptor and for transcriptional activation. Mol. Endocrinol., 9: 1166-1179.
8. Castle, J.C., 2011. SNPs occur in regions with less genomic sequence conservation. PLoS ONE, Vol. 6. 10.1371/journal.pone.0020660.
9. Chanock, S., 2001. Candidate genes and single nucleotide polymorphisms (SNPs) in the study of human disease. Dis. Markers, 17: 89-98.

10. Wright, S.I., 2005. The effects of artificial selection on the maize genome. Science, 308: 1310-1314.
11. Bali, V. and Z. Bebok, 2015. Decoding mechanisms by which silent codon changes influence protein biogenesis and function. Int. J. Biochem. Cell Biol., 64: 58-74.
12. Sauna, Z.E. and C. Kimchi-Sarfaty, 2011. Understanding the contribution of synonymous mutations to human disease. Nat. Rev. Genet., 12: 683-691.
13. Shabalina, S.A., N.A. Spiridonov and A. Kashina, 2013. Sounds of silence: Synonymous nucleotides as a key to biological regulation and complexity. Nucl. Acids Res., 41: 2073-2094.
14. Hyppönen, E. and B.J. Boucher, 2018. Adiposity, vitamin D requirements and clinical implications for obesity-related metabolic abnormalities. Nutr. Rev., 76: 678-692.
15. Zhang, Q., Y. Cheng, M. He, T. Li, Z. Ma and H. Cheng, 2016. Effect of various doses of vitamin D supplementation on pregnant women with gestational diabetes mellitus: A randomized controlled trial. Exp. Ther. Med., 12: 1889-1895.
16. Dean, A., K.M. Sullivan and M.M. Soe, 2013. OpenEpi: open source epidemiologic statistics for public health. https://www.OpenEpi.com
17. Holick, M.F., N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon and D.A. Hanley *et al*., 2011. Evaluation, treatment and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J. Clin. Endocrinol. Metab., 96: 1911-1930.
18. Bottero, D., M. Griffith, C. Lara, D. Flores and L. Pianciola *et al*., 2013. *Bordetella holmesiiin* children suspected of pertussis in Argentina. Epidemiol. Infect., 141: 714-717.
19. Chahil, J.K., K. Munretnam, N. Samsudin, S.H. Lye and N.A.N. Hashim *et al*., 2015. Genetic polymorphisms associated with breast cancer in Malaysian cohort. Indian J. Clin. Biochem., 30: 134-139.
20. Burton, A., D.G. Altman, P. Royston and R.L. Holder, 2006. The design of simulation studies in medical statistics. Statist. Med., 25: 4279-4292.
21. Crider, K.S., N. Whitehead and R.M. Buus, 2005. Genetic variation associated with preterm birth: A HuGE review. Genet. Med., 7: 593-604.
22. Larqué, E., E. Morales, R. Leis and J.E. Blanco-Carnero, 2018. Maternal and foetal health implications of vitamin D status during pregnancy. Ann. Nutr. Metab., 72: 179-192.
23. Grant, W.B., 2011. Adequate vitamin D during pregnancy reduces the risk of premature birth by reducing placental colonization by bacterial vaginosis species. mBio, Vol. 2. 10.1128/mBio.00022-11.
24. De-Regil, L.M., C. Palacios, L.K. Lombardo and J.P. Peña-Rosas, 2016. Vitamin D supplementation for women during pregnancy. Cochrane Database Syst. Rev., 2016: 1-94.
25. Wei, S.Q., H.P. Qi, Z.C. Luo and W.D. Fraser, 2013. Maternal vitamin D status and adverse pregnancy outcomes: A systematic review and meta-analysis. J. Maternal-Fetal Neonatal Med., 26: 889-899.
26. Qin, L.L., F.G. Lu, S.H. Yang, H.L. Xu and B.A. Luo, 2016. Does maternal vitamin D deficiency increase the risk of preterm birth: A meta-analysis of observational studies. Nutrients, Vol. 8. 10.3390/nu8050301.
27. Zhou, S.S., Y.H. Tao, K. Huang, B.B. Zhu and F.B. Tao, 2017. Vitamin D and risk of preterm birth: Up-to-date meta-analysis of randomized controlled trials and observational studies. J. Obstet. Gynaecol. Res., 43: 247-256.
28. Wagner, C.L., S.N. Taylor, A. Dawodu, D.D. Johnson abd B.W. Hollis, 2012. Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients, 4: 208-230.
29. Tamblyn, J.A., M. Hewison, C.L. Wagner, J.N. Bulmer and M.D. Kilby, 2015. Immunological role of vitamin D at the maternal–fetal interface. J. Endocrinol., 224: R107-R121.
30. Korf, H., B. Decallonne and C. Mathieu, 2014. Vitamin D for infections. Curr. Opin. Endocrinol. Diabetes Obesity, 21: 431-436.
31. Ibrahim, Z.M., E.H. madany, R.M.A. Aal and M.M. El Biely, 2013. Role of 1,25-dihydroxyvitamin D (vitamin D3) as immunomodulator in recurrent missed miscarriage. Middle East Fertil. Soc. J., 18: 171-176.
32. Evans, K.N., L. Nguyen, J. Chan, B.A. Innes, J.N. Bulmer, M.D. Kilby and M. Hewison, 2006. Effects of 25-hydroxyvitamin D3 and 1, 25-dihydroxyvitamin D3 on cytokine production by human decidual cells. Biol. Reprod., 75: 816-822.
33. Pérez-López, F.R., V. Pasupuleti, E. Mezones-Holguin, V.A. Benites-Zapata, P. Thota, A. Deshpande and A.V. Hernandez, 2015. Effect of vitamin D supplementation during pregnancy on maternal and neonatal outcomes: A systematic review and meta-analysis of randomized controlled trials. Fertil. Steril., 103: 1278-1288.e4.
34. Roth, D.E., M. Leung, E. Mesfin, H. Qamar, J. Watterworth and E. Papp, 2017. Vitamin D supplementation during pregnancy: state of the evidence from a systematic review of randomised trials. BMJ, Vol. 359. 10.1136/bmj.j5237.
35. Lechtermann, C., B.P. Hauffa, R. Herrmann, M.M. Schündeln, A. Gellhaus, M. Schmidt and C. Grasemann, 2014. Maternal vitamin D status in preeclampsia: Seasonal changes are not influenced by placental gene expression of vitamin D metabolizing enzymes. PLoS ONE, Vol. 9. 10.1371/journal.pone.0105558.
36. El-Beshbishy, H.A., M. Tawfeek, I. Taha, I. Sultan, T. Fadulelahi, A. Shaheen and G. Morsy, 2015. Association of vitamin D receptor gene BsmI (A>G) and FokI (C>T) polymorphism in gestational diabetes among Saudi Women. Pak. J. Med. Sci., 31: 1328-1333.
37. Manzon, L., G. Altarescu, A. Tevet, M.S. Schimmel, D. Elstein, A. Samueloff and S. Grisaru-Granovsky, 2014. Vitamin D receptor polymorphism FokI is associated with spontaneous idiopathic preterm birth in an Israeli population. Eur. J. Obstetrics Gynecol. Reprod. Biol., 177: 84-88.

38. Javorski, N., C.A.D. Lima, L.V.C. Silva, S. Crovella and J. de Azêvedo Silva, 2018. Vitamin D receptor (VDR) polymorphisms are associated to spontaneous preterm birth and maternal aspects. Gene, 642: 58-63.
39. Barchitta, M., A. Maugeri, M.L. Rosa, R.M.S. Lio and G. Favara *et al*., 2018. Single nucleotide polymorphisms in vitamin D receptor gene affect birth weight and the risk of preterm birth: Results from the "mamma and bambino" cohort and a meta-analysis. Nutrients, Vol. 10. 10.3390/nu10091172.
40. Dutra, L.V., F.A. Affonso-Kaufman, F.R. Cafeo, M.S. Kassai and C.P. Barbosa et al., 2019. Association between vitamin D plasma concentrations and VDR gene variants and the risk of premature birth. BMC Pregnancy Childbirth, Vol. 20. 10.1186/s12884-019-2671-2.
41. Baczyńska-Strzecha, M. and J. Kalinka, 2017. Assessment of correlation between vitamin D level and prevalence of preterm births in the population of pregnant women in Poland. Int. J. Occup. Med. Environ. Health, 30: 933-941.
42. Rosenfeld, T., H. Salem, G. Altarescu, S. Grisaru-Granovsky, A. Tevet and R. Birk, 2017. Maternal–fetal vitamin D receptor polymorphisms significantly associated with preterm birth. Arch. Gynecol. Obstet., 296: 215-222.