

AN ASSOCIATION BETWEEN THE VITAMIN D RECEPTOR BSMI POLYMORPHISM AND THE INCIDENCE OF PLACENTAL INSUFFICIENCY IN WOMEN AT HIGH RISK OF INFECTION

INTRODUCTION

Lately, the role of vitamin D (VD) in reproductive processes is being given increasing importance, due to the presence of its receptors in the vascular endothelium, uterus, ovaries, trophoblast cells and placenta [4, 9, 12, 17].

VD is a fat-soluble steroid vitamin that exerts its effect through the genomic pathway by binding to the intracellular vitamin D receptors (VDR) and an extra-genomic mechanism interaction of VD with its receptor which activates rapid signal transduction cascades. The VDR has ligand-binding and DNA-binding domains that bind to the VD and promoter sequences of VD-responsive genes. This data have provided new insight into the classical role of VD and its extraskeletal effects [6, 13].

It is known that besides kidney, the placenta is also the main site of synthesis of the active metabolite of VD due to the expression of CYP27B1 in the trophoblast and decidual tissue. Placental expression of CYP27B1 mRNA begins early in pregnancy and may play an important role in the placental response to infection [6].

The presence of VDR on some immunocompetent cells such as T-lymphocytes and macrophages, sparked off discussion about its potential role in the pathophysiologic mechanisms of some diseases such as rheumatoid arthritis and diabetes mellitus, as well as in the innate immune system and the pre-conception stage of pregnancy [8, 16, 17, 28].

Study of single nucleotide polymorphism of the gene encoding VDR, in particular studying the G63980A-polymorphism of the b/B allele (international code rs1544410), showed that this is characterized by the substitution of the nucleotide guanine (G) for adenine (A) in the non-coding gene region, including the BsmI-restriction site. In people of European origin, it is estimated to occur in about 30–40% of individuals and may be responsible for an increased risk of fractures, sporadic primary hyperparathyroidism, and other diseases [2, 3, 27]. However, polymorphism of VDR gene BsmI (A to G), is understudied, especially its possible role in obstetric complications.

To date, literature regarding the effect of the single nucleotide BsmI polymorphism on reproductive function, in particular pregnancy, has been rather scanty.

Objectives of the study. We tested the hypothesis whether the polymorphism of the gene encoding VDR, BsmI (A > G, rs1544410), is associated with the development of placental dysfunction (PD), as evidenced by abnormal Doppler readings on ultrasound, in pregnant women at high infectious risk (HIR). For the purposes of this study, HIR women were considered those having a positive urine culture, a positive nasopharyngeal, urogenital swab culture or polymerase chain reaction (PCR) result for a micro-organism or a positive TORCH screen.

MATERIALS AND METHODS

Study population and participants

An observational-analytical study was carried out according to the «case-control» principle of two groups of women:

- the main group included 56 pregnant women (I group), aged 18–40 with HIR and confirmed PD;
- the control group consisted of 40 apparently healthy women with physiological pregnancy and no evidence of intra-uterine infection (II group).

The study was conducted in Odessa, Ukraine (Maternity Hospital No. 1 and No. 5) over a two year period between 2018 and 2020. Special enzyme immunoassay and molecular genetic studies were carried out in “LLC Diagnostic Center Evgenika” in Odessa, Ukraine.

Inclusion criteria in the group were a pregnancy complicated by PD; age from 18 to 40 years and any clinical signs of intra-uterine infection.

The study excluded women under 18 or over 40 years of age as well as women with other co-morbid medical conditions.

Ethical approval

The study was approved by the Odessa National Medical University Bioethics Commission (protocol No. 124 from 02.02.2018) and in accordance with the principles of the WMA Declaration of Helsinki. All patients signed informed consent to participate in the study, conduct standard general clinical studies as well as confirm HIR and PD in accordance with the regulatory requirements of Ukraine.



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High infectious risk determination

Urogenital and nasopharyngeal swabs and urine were cultured for any bacteria, along with their sensitivities to antibiotics, and PCR diagnostics, including femoflor screen were used. The criterion for the risk of infection of the urogenital tract was the release of opportunistic microflora in the amount of 10^5 CFU/ml or more; bacteriuria is considered significant when the growth of bacterial cultures in the urine is $\geq 10^5$ CFU/ml, in two consecutive samples or in one portion of urine obtained with the help of a catheter.

An enzyme-linked immunosorbent assay (ELISA) test was used to diagnose TORCH infections (Toxoplasmosis, Other (infection), Rubella, Cytomegalovirus, Herpes simplex infection). If specific immunoglobulins were detected in a diagnostically significant titer, a repeat study was performed after 2 weeks to determine the avidity and affinity of antibodies. The diagnosis of TORCH infections was based on the determination of G/M class immunoglobulin (IgG, IgM) and antibody avidity in the blood over time.

The history of inflammatory chronic diseases of the gastrointestinal tract, urinary system, kidneys, acute respiratory viral infection during pregnancy was taken into account.

The diagnosis of intrauterine infection was further confirmed by ultrasound data, in particular, features such as oligo- and polyhydramnios, hypertrophy or hypoplasia of the placenta, calcification and placental oedema or fetal organ changes such as ventriculomegaly, hyperechoic intestine and hepatomegaly.

Placental dysfunction determination

To assess the hormonal function of the placenta, the levels of human chorionic gonadotropin (hCG) and estriol were determined by the ELISA method on the Cobas Integra 400 Plus analyzer (Roche Diagnostics, Switzerland). The condition of the intrauterine fetus was assessed by ultrasound with Doppler ultrasound of the utero-placental-fetal blood flow ("Samsung Medison UGEOWS80A"; Samsung Medison CO, LTD, 2014, Korea). The location of the placenta and its appearance were assessed. Examples of abnormal placental changes included the presence of hemorrhages, thickness and maturity appropriate for gestational age. Sonographically, the change in placental thickness correlates linearly with gestational age: this position has been used to identify placental hypertrophy or hypoplasia. According to the formula, the approximate gestational age (in weeks) is equal to the thickness of the placenta (in cm \pm 10 mm). The maximum thickness of a normal placenta at any point during pregnancy is often considered to be 4 cm [14, 26]. Cardiotocography was performed on fetal monitors "Sonicaid Team Care" (Huntleigh Healthcare Ltd, 2006, UK) with short term variation assessment and using the Fisher scale.

Total VD levels and PCR study determination of the VDR gene region for the presence of the BsmI polymorphism (rs1544410) mutant variation

The VD serum concentration was determined by ELISA method, based on the competitive binding principle, on a Cobas Integra 400 Plus analyzer (Roche Diagnostics, Switzerland) using the ELECSYS VD total II test system. The analytical sensitivity, calculated using the mean and 2 standard deviations was 2.89 ng/

ml; the coefficient of variation (CV) was 4.4, 3.0, and 6.6% at concentrations of 25.1, 43.2, and 93.7 ng/ml, respectively. Blood sampling from the vein was carried out in the morning, after an eight hour of fasting. The studies were conducted in the spring to avoid seasonal fluctuations in serum VD, during the period from 24 to 34 weeks of gestation when the diagnosis of PD was established.

In the first stage, 20 μ l of the serum sample was pretreated with reagents 1 and 2 to release 25-hydroxyvitamin D from VD binding protein (VDBP). Then the pretreated sample interacts with labeled ruthenium VDBP, resulting in the formation of a complex between 25-hydroxyvitamin D and ruthenylated VDBP. The reaction mixture was sucked into the measuring cell, where microscopic water particles were captured on the surface of the electrode. Unbound substances were then removed using the substrate-reagent Pro-Cell M. Applying voltage to the electrode subsequently caused a chemiluminescent radiation, which was measured by a photomultiplier. Results were determined using a calibration curve that was custom-generated using the 2-point calibration and a master curve provided by the reagent barcode. The "Clinical Practice in the Prevention, Diagnosis and Treatment of VD Deficiency: A Central and Eastern European Expert Consensus Statement" was used to define VD levels as sufficient if > 30 ng/mL, insufficient if ≥ 20 ng/mL but < 30 ng/mL and deficient if < 20 ng/mL [21].

DNA extraction from whole blood samples

Isolation of genomic DNA was performed from whole blood using the Proba-Rapid-Genetika reagent from "DNA-Technology" (Russia). The required number of plastic vials provided by "Scientific Specialties Inc." (USA) and each with a maximum capacity of 1.5 mls were correctly annotated according to the number of samples for analysis (study and control group participants together) and with a negative control sample "K". 600 μ l of lysing solution was added to each labeled tube. A 100 μ l sample of peripheral blood was added to each test tube except for the control sample tube, to which 100 μ l sterile saline was added. The lids of the vials were closed and these were shaken for 3–5 s using a "Microspin" FV-2400 vortex machine manufactured by "Biosan" (Latvia). The tubes were centrifuged using a Sigma 1–14 centrifuge manufactured by "Sigma Laborzentrifugen" (Germany) at 13,000 rpm for 1 min to release the DNA-protein complex as a precipitate. The supernatant was discarded and the precipitate added to Thermostat test tubes at 98 °C for 10 min in thermostat "Gnome" company "DNA-technology" (Russia). 300 μ l of Proba-Rapid-Genetika reagent ("DNA-Technology", Russia) was added to the precipitate and the vials were shaken on a Vortex machine for 5–10 s. The addition of buffer solution to the precipitate caused the dissociation of proteins from DNA and for a highly purified form of DNA to be released into solution. The tubes were centrifuged at 13,000 rpm for 3 minutes. This produced a supernatant containing isolated DNA ready to be added to the reaction mixture for a real time polymerase chain reaction.

Real-time PCR

The principal feature of the real-time PCR mode is monitoring and quantitative analysis of the accumulation of PCR products, as well as automatic registration and interpretation of the results obtained. Sample genotyping was performed using

kits from "DNA Technology" (Russia), microtubes from Greiner-bio-one (USA), and a DT-96 detection cyler from "DNA Technology" (Russia). The genotyping results were determined automatically using the software from the DT-96 detection cyler.

Five ml blood sample was collected in EDTA-tube. DNA samples were all quoted and stored at -20 °C till analysis. Two DNA probes were introduced into the reaction mixture (for two alleles of the studied polymorphism – A > G), each of which contains a fluorescent dye at the 5'-end and a fluorescence quencher at the 3'-end. In the initial solution, background fluorescence is minimal, but as a result of amplification, fluorescent dyes are released, which makes it possible to detect the presence of one or another allele of a DNA sample in the amplified mixture. Restriction fragment length polymorphism (RFLP-PCR) was used to identify VDR genotype. Amplified PCR product (10 ml) was digested (37 °C for 20 h) with 4 U of BsmI restriction enzyme in 20 µl reaction volume. Digested product was electrophoresed on 2% agarose gel. In this study we used forward and reverse primers: (F5'-ACCAAGACTACAAGTACCGCGTCA-3' and R5'-CTCCTCTCCACCTCTAACCGC-3').

Genotype was determined according to fragments length i.e. homozygote GG(BB) subjects = 650 and 172bp product; heterozygote GA (Bb) subjects = 822, 650 and 172bp products and homozygote AA (bb) subjects = 822bp product [23].

The reaction mixture consisted of the following reagents; Taq polymerase with 5'-nuclease activity (dissolved in buffer: 10 mM HEPES; pH 8.0; 100 mM KCl; 1% Tween-20; 5 mM DTT; 50% glycerol), PCR mix (670 mM Tris-HCl (pH 8.8 at 25 °C), 0.1% Tween-20, 2 mM dNTPs, 10 mM primers, 5 mM probes, deionized water and the DNA sample.

Statistical analysis

Result analysis was done using Microsoft Excel 2007 and Biostat, Statistica 6.0 software from Install Shield Software Corporation for Windows (USA).

Baseline and resurvey sample characteristics were presented as mean (S.D.) or percentage. The ANOVA test was used to examine between group differences in baseline characteristics for continuous variables and the χ^2 test for dichotomous measures.

The quantitative variable results were checked for normality distribution using the Shapiro-Wilk test. The Student's t-test was used to analyze the variables which are normally distributed, whereas the Mann-Whitney U-test was used to analyze the non-normally distributed data. Differences were considered statistically significant at $P < 0.05$ (95% significance level) and at $P < 0.01$ (99% significance level).

Differences in genotype distribution and consistency of genotype distribution with Hardy Weinberg Equilibrium (HWE) were tested using the Chi-Squared test (if p was higher than 0.05, the sample of this genotype corresponded to the HWE) According to HWE, genetic variability in a population will remain constant from one generation to the next in the absence of disturbing/variable factors [22, 24]. The various alleles' frequency indicators distribution and genotypes statistical significance in the groups and their possible influence on the phenotypic compared groups characteristics was determined by calculating the odds ratio (OR) using a 95% confidence interval (CI).

The relationship between the studied parameters was determined by correlation analysis using Spearman's rank correlation coefficient (R) with subsequent significance establishment by the t criterion. In this case, the correlation coefficient was considered to be moderate from 0.3 to 0.7 and strong, more than 0.7. The error probability calculation and Fisher's test (qualitative indicators) made it possible to assess the differences' reliability between the mean samples values and the detected data correlation.

RESULTS

Characteristics of the study population

All patients' demographic data and clinical characteristics presents in Table 1. The data obtained indicate a higher frequency of pregnancy complications in the I group. There was heterogeneity in parity between the main and control groups. The mean weight of newborns in the I group (3299.11 ± 128 g) was significantly lower than in the II group (3643.24 ± 136 g; $t = 4.17$; $p < 0.01$); significant differences were also observed in neonatal growth rates (54.25 ± 3.7 vs 52.41 ± 3.6 cm; $t = 3.97$; $p < 0.01$).

A correlation analysis between the weight of newborns and the level of VD showed a significant direct relationship both in the I ($r_s = 0.707$) and II groups ($r_s = 0.427$, $p < 0.01$). The fetal growth retardation (FGR) was diagnosed only in 7.14% of women from the I group. The average level of serum VD in these women was 15.05 ± 2.4 ng/ml, which corresponded to a pronounced VD deficiency. Apgar score assessment of newborns showed significant differences between the groups (7.02 and 8.78 points respectively; $t = 5.16$; $p < 0.01$).

There was a high frequency of extragenital inflammatory diseases in I group, changes in morphometric parameters and pathological location of the placenta (placental hypertrophy – 4.3 times, low placentation – 3.6 times more). Only in the I group were fetal ventriculomegaly (12.5%) and hyperechoic intestine (10.7%) detected. Amnion pathology was also diagnosed with greater frequency in patients of this group (polyhydramnios – 2.8 times, oligohydramnios – 7.8 times).

Figure 1 showed that the serum VD average level in patients with PD was reliably lower than in patients with physiological pregnancy (1.4 times, $F = 0.0258$; $p < 0.01$).

The alleles frequency distribution of the VDR gene BsmI polymorphism (rs1544410) in pregnant women with HIR and PD

The molecular genetic testing results showed that there was no significant difference in the frequency of homozygous AA individuals between I and II groups respectively (10.7 vs. 20%, $P > 0.05$). The GG genotype was more common in the control group (18 out of 40 women and 10 out of 56 in the main group, $P \leq 0.01$) (Figure 2).

The AG heterozygous combination was observed with a reliably higher frequency in the I group (40 out of 56 women – 71.4%), as opposed to the II group (14 out of 40 women – 35%; $P < 0.01$). A direct positive correlation was found between the PD frequency and the AG genotypic expression (OR = 3.8; 95% CI 2.1 – 6.8; $\chi^2 = 20.88$; $P < 0.01$). These findings are summa-

Table 1. Baseline characteristics of the population studied

Index	Main group n = 56	Control group n = 40	Reliability
Age, years	29.2 ± 4.3	30.4 ± 3.12	P > 0.05
Body mass index, kg/m ²	22.2 ± 1.7	22.8 ± 1.93	P > 0.05
Primiparous, %	71.4	55	F = 0.028; P < 0.05
Multiparous, %	28.6	45	F = 0.028; P < 0.05
Gestational age at delivery, weeks	38.01 +/- 1.21	38.63 +/- 1.01	
VD levels, ng/mL	30.6 +/- 9.6	43.5 +/- 13.3	F = 0.0258; p < 0.01
Preeclampsia, % / absolute number	14.3 / 8	0	F = 0.0007; P < 0.05; $\chi^2 = 6.23$; P < 0.05
Intrauterine growth retardation, % / absolute number	7.2 / 4	0	F = 0.014; P < 0.05
Anemia, % / absolute number	50 / 28	10 / 4	F = 0.00001 P < 0.05; $\chi^2 = 12.19$; P < 0.01
Acute respiratory viral infection, %, absolute number	30.7 / 17	7.5 / 3	F = 0.0004; P < 0.05; $\chi^2 = 7.39$; P < 0.01
Chronic / gestational pyelonephritis, % / absolute number	37.5 / 21	15 / 6	F = 0.0006; P < 0.05; $\chi^2 = 5.84$; P < 0.05
Chronic tonsillitis, % / absolute number	16.1 / 9	5 / 2	F = 0.0096; P < 0.05
Chronic gastritis / cholecystitis, % / absolute number	8.9 / 5	5 / 2	F = 0.007; P < 0.05
Bacterial vaginosis % / absolute number	32.1 / 18	7.5 / 3	F = 0.0008; P < 0.05; $\chi^2 = 8.29$; P < 0.01
Polyhydramnios, % / absolute number	21.4 / 12	7.5 / 3	F = 0.015; P < 0.05
Oligohydramnios, % / absolute number	39.3 / 22	5 / 2	F = 0.00001; P < 0.05
Low placentation, % / absolute number	26.8 / 15	7.5 / 3	F = 0.0006; P < 0.05
Placental hypertrophy, % / absolute number	10.7 / 6	2.5 / 1	F = 0.0245; P < 0.05
Small size placenta, % / absolute number	3.6 / 2	0	F = 0.0007; P < 0.05; $\chi^2 = 6.23$; P < 0.05
Premature placental maturation, % / absolute number	7.1 / 4	0	F = 0.0007; P < 0.05; $\chi^2 = 6.23$; P < 0.05
Fetal ventriculomegaly, % / absolute number	12.5 / 7	0	F = 0.0002; P < 0.001;
Hyperechoic intestine in the fetus, % / absolute number	10.7 / 6	0	F = 0.0007; P < 0.01;

Note: data are presented as mean ± S.D. for continuous and percentage for categorical variables.

rized in Table 2. An inverse correlation dependence of average strength was revealed between the frequency of the GG genotype and the frequency of PD (OR = 0.27; 95% CI 0.15 – 0.51; $\chi^2 = 16.71$; p < 0.01).

Genotypic distribution of the VDR gene BsmI polymorphism in the main and control groups according to the HWE

Most (87.5%) of pregnant women with PD and insufficient VD (below 30 ng/ml) were carriers of the AG genotype as opposed to the control group, where there were no women with VD insufficiency that were carriers of such genotype (F = 0.00001; P < 0.01). Among women in the II group with insufficient VD, 83.3% of women were homozygous with the GG genotype, as opposed to the I group where there were no women with such a genotype (F = 0.00001; P < 0.01). The AA genotype was the least common for both groups respectively (10.7% and 20%, F = 0.553; P > 0.05). These results are summarized in Table 3.

A significant difference was found between the incidence of all genotype variants in the I group and their expected frequencies in the population using HWE. The frequency of the homozygous genotype GG in the main group was 17.8

vs. 28.7% of the expected, the incidence of the homozygous AA genotype was 10.7 vs. 21.6%, and the heterozygous AG genotype, the commonest genotype in this group, was 71.4 vs. 49.8% ($\chi^2 = 10.64$, P < 0.01).

The HWE in the control group showed the following results. The incidence of heterozygous women with the AG genotype was 35% versus the expected 46.9%, that of the GG genotype was 45 vs. 39.1%, and the AA genotype incidence was 20% vs. 14.1%. Differences between actual and expected incidences were not found to be statistically significant for all genotypes ($\chi^2 = 2.5671$, P > 0.05). These results are summarized in Table 4.

Main Results

Pregnant women with PD on the background of HIR have lower serum VD levels than women having uncomplicated pregnancies. The single nucleotide BsmI polymorphism of the gene encoding VDR in pregnant women carrying the heterozygous AG genotype probably causes a greater risk of developing PD in conditions of VD deficiency. This seems to suggest an association between low VD levels, the BsmI single nucleotide polymorphism in the VDR and the development of PD. In accordance with HWE, in women with the AG genotype, the risk of developing PD is higher than the expected population risk.

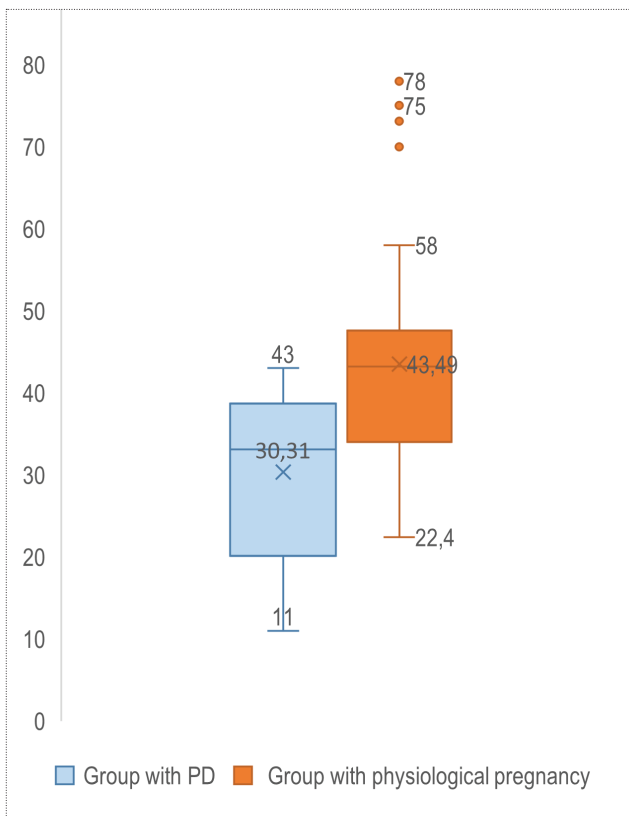


Figure 1. The average level of 25(OH)D (mean \pm SD, ng/ml) in women with placental dysfunction and in women with physiological pregnancy. VD concentration and pregnant women's vitamin D-status, determined by ELISA, shows that the average content of VD in pregnant women group with placental dysfunction and high infectious risk (30.3 ± 9.6 ng/ml) was reliably less than in group with physiological course of pregnancy without any infectious agents influence, (43.5 ± 13.3 ng/ml, $F = 0,0258$; $p < 0,01$).

Strengths and Limitations

The VD status determination in pregnant women with PD and HIR compared to healthy pregnant women was carried out for the first time. This is also the first study to our knowledge, to examine the relationship between the BsmI polymorphism and PD. For the first time, the law of genetic balance, HWE was implemented in the study of VDR gene polymorphism in pregnant women with PD, which is important in terms of predicting and preventing this pregnancy complication. The advantages of the study also include the use of the HWE to determine the frequency of distribution of genotypes in the population, as well as ANOVA analysis of variance to study differences between groups.

One major limitation is perhaps, the study of only one of the genes encoding VDR BsmI polymorphism type. Since a number of other polymorphisms are known such as the Apal, and TagI, polymorphisms, their investigation could probably enhance the clinical significance of the study. The study population is also quite small, which limits the power of the study and hence warrants some caution in the results' interpretation. Hardy-Weinberg disequilibrium situations occur quite rarely, are usually the consequence of some external factor operating in natural selection and large numbers would be needed to prove such an effect. The over-representation of heterozygotes in both control and main groups might be causing confounding of the results due to cross-contamination, hence requiring caution in interpretation.

DISCUSSION

Publications by several medical societies such as the "AME and AACE Position Statement: Clinical Management of Vitamin D Deficiency in Adults" [5] have recommended a lower cut-off for vitamin D in the blood of pregnant women

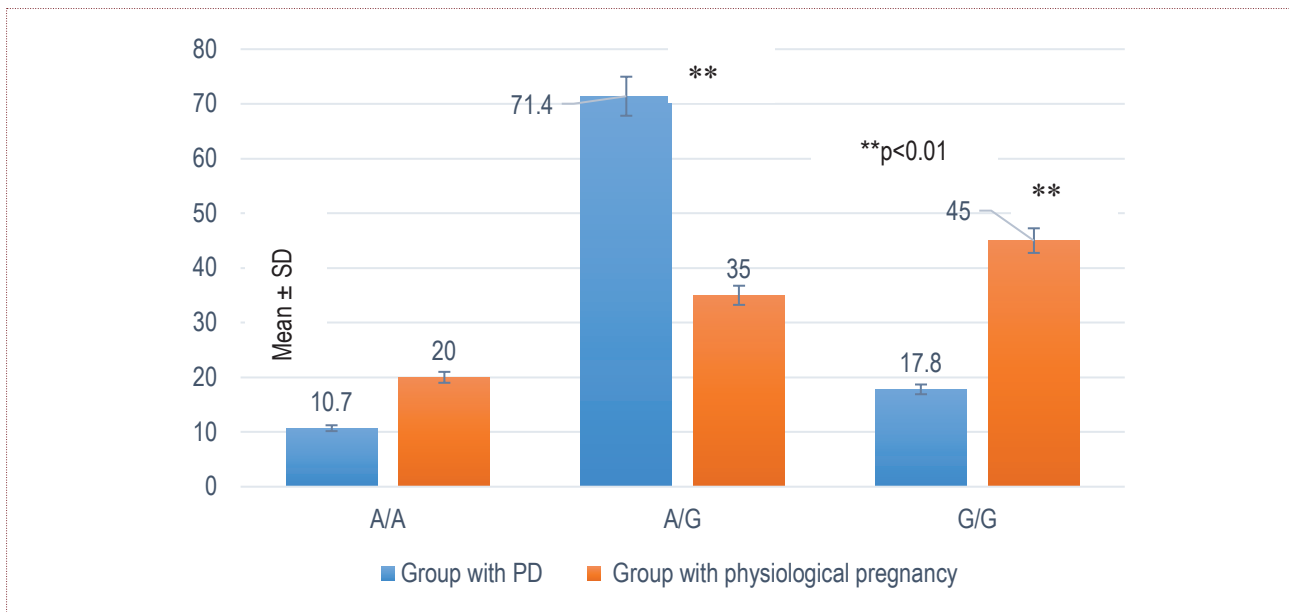


Figure 2. Variants of genotypes of BsmI polymorphism of the gene encoding VDR (%) A real-time PCR study results of the VDR gene region for the BsmI (rs1544410) polymorphism mutant presence showed that 10.7% of women with PD and HIR (n = 56) background and 20% of women with physiological pregnancy (n = 40) are homozygous carriers of the AA allele ($P \geq 0.05$) and the G allele, respectively, 17.8% and 45%, ($P \leq 0.01$). A heterozygous combination of the AG allele was found in 71.4% of pregnant women with PD and in 35% of women of the control group (** $P < 0.01$).

Table 2. Genotype and allele frequencies of VDR polymorphisms in the main and control groups

Genotypes	Main group, n = 56		Control group, n = 40		OR (95%CI)	χ ²	P
	Absolute number	%	Absolute number	%			
G/G	10	17.8	18	45	0.27 (0.15–0.51)	16.71	< 0.01
A/G	40	71.4	14	35	3.95 (2.19–7.1)	20.88	< 0.01
A/A	6	10.7	8	20	0.68 (0.31–1.48)	1.013	> 0.05
A	52 (of 112)	46.4	28 (of 80)	35	1.58 (0.89–2.79)	2.51	> 0.05
G	60 (of 112)	53.6	52 (of 80)	65	0.63 (0.36–1.12)	2.51	> 0.05

Table 3. Genotypic distribution of individuals with insufficient or deficient VD levels (< 30 ng/mL) in the main and control groups

Genotype	Women with low VD levels (< 30 ng/mL, %)		Reliability
	Group with PD	Control group	
A/A	10.70%	20%	F = 0.553; P > 0.05
A/G	87.50%	0%	F = 0.00001; P < 0.01
G/G	0%	83.30%	F = 0.00001; P < 0.01

Table 4. The Expected Incidence of the genotypes according to HWE compared to their actual incidences in the main and control group

BsmI polymorphism genotypes of VDR	Expected Incidence		Actual Incidence		χ ²	P
	Absolute number	%	Absolute number	%		
Main group						
Common homozygotes – G/G	16.1	28.7	10	17.8	10.64	P < 0.01
Heterozygotes – A/G	27.1	49.8	40	71.4		
Rare homozygotes – A/A	12.1	21.6	6	10.7		
Total	56	100	56	100		
Control group						
Common homozygotes – G/G	15.6	39.1	18	45	2.5671	P > 0.05
Heterozygotes – A/G	18.6	46.8	14	35		
Rare homozygotes – A/A	5.6	14.1	8	20		
Total	40	100	40	100		

of 40 ng/mL, suggesting that a number of pregnancy complications such as preterm birth, preeclampsia and gestational diabetes may be associated with VD deficiency [4, 18]. The FGR risk in pregnant women with VD deficiency is 6 times higher than in women with its optimal level. According to the authors under conditions of VD deficiency abnormal apoptosis of trophoblast cells is observed, which leads to placental functional failure [1].

We found that in pregnant women with PD and HIR, the serum VD level is 1.4 times lower than in healthy women. There is also a certain clinical parallel between the placental dysfunction and placental morphological changes such as hypertrophy of the placenta and low placentation. In a number of studies, these morphological changes are regarded as clinical manifestations of pathological placentogenesis and predictors of PD, preeclampsia and fetal intrauterine growth retardation [10, 19].

The immunobiological role of VD is associated with the pathophysiology of some diseases characterized by a systemic inflammatory response [8, 28]. According to the literature, there

is no direct relationship between the severity of the infectious process and fetal teratogenesis or dysmorphogenesis [7, 20, 25].

One of the criteria for selecting women into the main group was the presence of a HIR. Based on the results of the study and literature data, it is quite difficult to determine whether the presence of an infectious factor is a consequence of a primary VD deficiency or whether the VD deficiency is secondary to infection. A high incidence of bacterial vaginosis, acute respiratory viral infection and inflammatory diseases of the kidneys were all noted in the study group, all of which can contribute to the formation of PD [8, 11, 15].

Our study showed that the PD developing probability in heterozygous carriers of the mutant BsmI polymorphism (rs1544410) with the AG genotype is twofold higher (OR = 3.95, 95% CI 2.19–7.1, χ² = 20.88, P < 0.01), than in homozygotes with the AA or GG genotype carriers did not develop PD even under suboptimal blood VD levels. The insignificant frequency of the AA genotype in the groups probably indicates that this variant does not affect the phenotypic characteristics of women and

the course of the pregnancy, despite VD deficiency. According to H. Wolski et al., (2021), in women with recurrent miscarriages were observed in VDR BsmI polymorphism (GG vs. GA and AA, OR = 0.56, p = 0.036 and OR = 1.49, p = 0.035, respectively. M. Alzaim et al. (2022) used to determine FokI VDR genotype in pregnant Arab women and found that carriers of the ff allele had a significant risk for full maternal metabolic syndrome (OR 4.2, 95% CI 1.4–12.2; adjusted p = 0.009) [3, 27]. These and other studies indicate the significance of genetic variants in VDR function, disturbed VD function and in complicated pregnancy [17, 19].

Each population has its own allele pool and a different frequency of unfavorable alleles: HWE allows assessing the population risk of genetically determined diseases. Phenotypic characteristics of an organism are formed under the influence of nonmodifiable genetic factors and modifiable environmental factors. HWE shows the expected patterns of phenotype formation depending on the genotype under the conditions of an ideal population and the absence of any mutations [20]. According to our data, among pregnant women with PD and HIR, the frequency of heterozygotes with the AG genotype exceeded the expected frequency in the population by 1.4 times.

It is possible that VDR gene BsmI polymorphism and VD deficiency in pregnant women may be the factors that increase the PD development likelihood in women with HIR.

One plausible mechanism by which this may occur could be that VD is one of the cofactors needed for the establishment

of a well vascularized placenta. The presence of the BsmI polymorphism could be contributing to a diminished response to VD, hence leading to PD.

CONCLUSIONS

According to the study, VD deficiency may increase the risk of PD. The BsmI (A > G) VDR polymorphism suggests its probable involvement in the development of PD in women at high infectious risk. In the context of VD deficiency in heterozygous polymorphic AG individuals PD was found to be 3.6 times commoner than in women with other genotype variants; the PD risk was minimal in homozygous women with the GG genotype.

Further research is needed to investigate the effects of VD on pregnancy, including molecular genetics. More studies are needed to investigate any associations related to the other VD receptor polymorphisms as well as the CYP27B1 and CYP24A1 gene polymorphisms. The possibility of predicting and preventing pregnancy complications at the preclinical stage by influencing modifiable risk factors may be a promising scientific venture.

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Conflict of Interests

None.

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AN ASSOCIATION BETWEEN THE VITAMIN D RECEPTOR BSMI POLYMORPHISM AND THE INCIDENCE OF PLACENTAL INSUFFICIENCY IN WOMEN AT HIGH RISK OF INFECTION

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Research objectives: the study examined the effect of the BsmI polymorphism of the gene encoding vitamin D receptors (A > G, rs1544410) on human placental dysfunction.

Materials and methods. An observational-analytical study carried out according to the «case-control» principle. The study was conducted in two maternity hospitals in the city of Odessa, Ukraine over the same period of time. Special enzyme immunoassay and molecular genetic studies were carried out at the LLC «Diagnostic Center Evgenika» in Odessa.

The intervention group included a total of 56 pregnant women aged 18 to 40 with high infectious risk and signs of placental dysfunction. The control consisted of 40 apparently healthy women with an uncomplicated pregnancy. The 25(OH)D blood level was determined by the ELISA method whereas the gene polymorphism encoding vitamin D receptors was determined by PCR.

Results. The number/percentage of women with placental dysfunction in the intervention and control groups respectively, their hydroxyvitamin D levels as well as the genotypes expressed was investigated. In placental dysfunction patients, vitamin D level was significantly lower than in healthy individuals. There was a direct correlation between the placental dysfunction frequency and the A/G genotype. In vitamin D deficiency, the heterozygous A/G genotype for vitamin D receptor gene polymorphic locus increased the risk of development of placental dysfunction by 3.6 times.

Conclusions. Vitamin D deficiency may increase the risk of placental dysfunction. For a more complete understanding between vitamin D receptor gene polymorphism and placental dysfunction, it is necessary to study all four vitamin D receptors polymorphisms with 25(OH)D levels correlations as well as polymorphisms of genes CYP27B1 and CYP24A1.

Keywords: placental dysfunction, vitamin D receptors, BsmI gene polymorphism.

ЗВ'ЯЗОК МІЖ BSMI ПОЛІМОРФІЗМОМ РЕЦЕПТОРА ВІТАМІНУ D ТА ЧАСТОТОЮ ПЛАЦЕНТАРНОЇ НЕДОСТАТНОСТІ В ЖІНОК ІЗ ВИСОКИМ ІНФЕКЦІЙНИМ РИЗИКОМ

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Мета дослідження: проаналізувати вплив BsmI поліморфізму гена, що кодує рецептори вітаміну D (A > G, rs1544410), на плацентарну дисфункцію.

Матеріали та методи. Спостережно-аналітичне дослідження проведено за принципом «випадок-контроль». Дослідження виконано на базі двох пологових будинків міста Одеси (Україна) протягом однакового періоду часу. Імуноферментні та молекулярно-генетичні дослідження проводились на базі ТОВ «Діагностичний центр Євгеніка» в Одесі.

До основної групи увійшли 56 вагітних жінок віком від 18 до 40 років із високим інфекційним ризиком та ознаками плацентарної дисфункції. До групи контролю увійшли 40 практично здорових жінок із неускладненим перебігом вагітності. Рівень 25(OH)D у крові визначався методом імуноферментного аналізу, а поліморфізм генів, що кодуєть рецептори вітаміну D, – методом полімеразної ланцюгової реакції.

Результати. Порівнювалась кількість/відсоток жінок із плацентарною дисфункцією в основній та контрольній групах відповідно, рівні гідроксिवітаміну D у сироватці крові в групах, а також частота варіантів BsmI поліморфізму гена, що кодує рецептори вітаміну D. У хворих на плацентарну дисфункцію рівень вітаміну D був достовірно нижчим, ніж у здорових осіб. Встановлено прямий кореляційний зв'язок між частотою плацентарної дисфункції та генотипом A/G. За дефіциту вітаміну D гетерозиготний генотип A/G за поліморфним локусом гена рецепторів вітаміну D збільшував ризик розвитку плацентарної дисфункції в 3,6 рази.

Висновки. Дефіцит вітаміну D може збільшити ризик дисфункції плаценти. Для повнішого розуміння зв'язку між поліморфізмом гена рецепторів вітаміну D і дисфункцією плаценти необхідно вивчити всі чотири поліморфізми рецепторів вітаміну D із кореляцією рівнів 25(OH)D, а також поліморфізми генів CYP27B1 і CYP24A1.

Ключові слова: плацентарна дисфункція, рецептори вітаміну D, поліморфізм гена BsmI.