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Original Research Article

Association of rs1544410 Polymorphism VDR gene with Recurrent Pregnancy Loss in Iraqi Patients Women

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Abstract: ***Objective*:** Recurrent loss of pregnancy is one of among the most prevalent clinical events that occur in the initial, second, and thrid trimesters. Therefore, while this research aims to assess the connection between the vitamin D receptors gene (V.D.R) SNPs (rs1544410) and the risk of recurrent pregnancy loss. ***Methods***: The cross-sectional research focused on fifty women with recurrent pregnancy loss and 50 control women without history of pregnancy failure. We applied the polymerase chain reaction (P.C.R) techniques to amplify the polymorphism areas of the V.D.R gene on each chromosome 12. The P.C.R products of every specimen were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, USA) and forwarded for sequencing using the Sanger sequencing method at Macrogen, Korea. All DNA sequences were compared to the reference genome sequence of the VDR gene. ***Results*:** Our results showed the case patient group consisted of 15(30 %) CC wild type, 29(58%) CT mutant heterozygote, and 6(12%) TT homozygote genotypes of rs1544410 in VDR gene, while the control included 25(50%) CC wild type, 21(42%) CT heterozygote, and 4(8%) TT homozygote genotypes. There was a significant decrease in CC genotype in recurrent pregnancy compared with control group of dominant model (P=0.04), and significant association of rs1544410 polymorphism and VD3, WBC, RBC, GOT, GPT and Diastolic Blood Pressure. ***Conclusion*:** The present research found a substantial association between rs1544410 variations of the V.D.R gene and recurrent abortion, and significant association of rs1544410 polymorphism with study parameters.

Keywords: rs 1544410, VDR Gene, Recurrent Pregnancy Loss, Polymorphism.

INTRODUCTION

Repeated pregnancy loss (R.P.L) has been defined in the USA as a minimum of two consecutive unsuccessful clinical pregnancies validated by ultrasonography or histology. RPL is one of the most difficult and aggravating challenges in reproductive medicine, causing significant emotional distress for patients, their families, and healthcare providers. When the underlying etiology remains unidentified, it can lead to anxiety and uncertainty for patients. R.P.L can be classified into primary and secondary types. Primary R.P.L refers to pregnancy loss in women with no previous live birth, while secondary RPL occurs in women who have had at least one live birth [1]. Vitamin D plays a vital role in regulating the immune system. During pregnancy, its deficiency is associated with several adverse outcomes, including preeclampsia, gestational diabetes mellitus (GDM), fetal growth restriction (FGR), preterm birth, and spontaneous abortion [2, 3]. Recent meta-analyses and cohort studies have demonstrated that low maternal serum vitamin D levels are significantly correlated with increased risk of miscarriage and poor pregnancy outcomes [4, 5]. Vitamin D is a steroid hormone that exerts its biological functions through the vitamin D receptor (VDR), a ligand-dependent transcription factor predominantly located in the cell nuclei. V.D.R acts as a genomic mediator of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and has been detected in various tissues, including reproductive organs such as the ovaries, endometrium, uterus, and placenta [6, 7]. The expression of VDR in

reproductive tissues suggests its potential involvement in essential reproductive processes such as folliculogenesis, implantation, and embryo development. Studies have shown that VDR expression is upregulated during the implantation window, indicating a role in enhancing endometrial receptivity [8]. Structural and functional analysis of the VDR protein has revealed distinct regions involved in DNA binding, ligand binding, receptor dimerization, and gene transactivation. VDR can be found in both the nuclei and cytoplasm of granulosa cells in ovarian follicles, further indicating its critical role in mediating the effects of vitamin D within the ovary [6-9]. Genetic variations within the VDR gene—referred to as single nucleotide polymorphisms (SNPs)—are common in the general population and may influence individual responses to vitamin D [10]. Notably, most research has focused on VDR polymorphisms in the 3′ and 5′ regions of the gene, particularly ApaI (rs7975232), TaqI, BsmI, and FokI. ApaI polymorphism, located in intron 8, involves a guanine-to-thymine (G→T) substitution that may alter VDR gene function and expression [10, 11].

This study aimed to investigate the relationship between VDR gene polymorphisms (rs 1544410) and recurrent pregnancy loss in Irqi patients women.

MATERIAL AND METHODS

Sample Collection

This cross-sectional study was conducted on 100 volunteers. The mean age of everyone who participated ranged from twenty to forty-five years. Fifty women experience unplanned pregnancy loss with history of three or more pregnancy failure. The control categories comprise 50 healthy women with no prior incidents of pregnancy loss. The criteria for exclusion included any prior experience of abortion, heart disease, coagulative and other chronic conditions (the renal, hepatic, and rheumatologic), multifetal gestations, molar pregnancies, thrombophilia, lupus, anti-phospholipid antibody syndrome, and other known reasons for spontaneous abortion. A daily vitamin D level of less than thirty was classified as insufficient.

Ethical Considerations

This study was conducted in accordance with ethical principles to ensure the rights and well-being of all participants. A total of 100 pregnant women were enrolled, including 50 patients diagnosed with gestational diabetes mellitus (GDM), some of whom had a history of recurrent miscarriage, and 50 healthy controls. Prior to sample collection, written informed consent was obtained from each participant after explaining the purpose and procedures of the study.

Confidentiality and privacy of participant information were strictly maintained throughout the research process. The study protocol was reviewed and approved by the relevant institutional ethics committee, ensuring compliance with international ethical standards for research involving human subjects.

Genetic Analysis and Genotyping

Genomic D.N.A of participants was extracted from leukocytes from the entire the blood by the salt-saturation procedure as earlier instructed (Miller *et al.,* 1988). Polymerase chain reaction (P.C.R) was employed for amplifying D.NA. Fragments of the polymorphism's regions on chromosomes. To achieve this, 415 bp DNA segments containing rs1544410 polymorphism were amplified utilizing one pair of previously developed primers for every variant. (Manzon *et al*., 2014). (Table 1).

Table 1: The sequence of applied primers to amplifying parts of VDR gene

Species	SNP	Target gene	Primer name	5'-3'	PCR size
Homo sapiens	rs1544410	VDR	F R	GAAGGACAAAGACCTGCTGAG ACCTCTAACCAGCGGAAGA	415pb

According to the findings of Gene sequencing, patients were categorized into three categories associated V.D.R gene polymorphism: normal (wild type), heterozygote, and homozygote. While extensively evaluating all people for heterozygote and homozygote status, data were evaluated utilizing sequenced. The results were subsequently assessed utilizing chi-square and independent t-tests. P-value of the 0.05 was deemed as statistically significant.

Statistical Analysis: Statistical analysis was done using Spss software and Microsoft Excel.

RESULTS

Table 2: Association of rs1544410 genotypes with study groups

Groups	Frequency n (%)		Control versus Abortion	
	Patient N=50(%)	Control N=50(%)	OR(CI)	χ2 \p-value
Genotype				
CC	15(30)	25(50)	2.33 (1.00 to 5.36)	4.17\0.04*
CT	29(58)	21(42)	0.524 (0.246 to 1.17)	2.56\0.11
TT	6(12)	4(8)	0.638 (0.193 to 2.32)	0.444\0.50
χ2				

Allele				
C	59(59)	71(71)	1	
T	41(41)	29(29)	1.70 (0.957 to 3.11)	3.16\0.08

* = significance under ($p<0.05$)

The result in table 2 showed significantly decreasing in the frequency cc genotype at patient group with risk compared with control (30% *versus* 50%, χ^2 4.17, p= 0.04, OR = 9.33). Other genotype showed no significant.

Table 3: Genotypes and Allele frequency of rs1544410

Genetics models	Genotypes	Abortion N=50 N (%)	Control N=50 N (%)	Control versus. Abortion	
				OR (95% CI)	χ2/ p-value
Codominant	CC	15(30)	25(50)	1	
	CT	29(58)	21(42)	2.30 (1.01 to 5.28)	3.74\0.05
	TT	6(12)	4(8)	2.50 (0.684 to 8.71)	1.66\0.20
Dominant	CC	15(30)	25(50)	1	
	CT+TT	35(70)	25(50)	2.33 (1.00 to 5.36)	4.17\0.04*
Recessive	CC+CT	44(88)	46(92)	1	
	TT	6(12)	4(8)	0.638 (0.193 to 2.32)	0.444\0.5
Over- dominant	CC+TT	21(42)	29(58)	1	
	CT	29(58)	21(42)	1.91 (0.853 to 4.07)	2.56\0.11
Allele frequency					
Alleles	C	59(59)	71(71)	1	
	T	41(41)	29(29)	1.70 (0.957 to 3.11)	3.16\0.08

The result in (table 3) showed the analysis of the rs1544410 polymorphism in the (VDR) gene revealed a statistically significant association only in the dominant genetic model, where carriers of the T allele (CT + TT genotypes) were at a higher risk of recurrent miscarriage compared to individuals with the CC genotype. The odds ratio (OR) was 2.33, with a 95% confidence interval (CI) of 1.00–5.36, and a p-value of 0.04. In contrast, the other genetic models (codominant, recessive, and over-dominant) did not show statistically significant differences ($p < 0.05$). However, further studies with larger sample sizes are recommended to confirm this association and provide functional biological insights.

Table 4: The genotype and parameters differences of VDR gene polymorphism (rs1544410) in the Abortion cases group compared to the control group

Parameters	Groups	Genotypes Mean±SD			*p*-value
		CC	CT	TT	
VD3	Control	36.02± 4.807Aa	32.47 ± 3.302Ab	38.30± 6.487Aab	0.0090**
	Abortion	12.42± 4.249Ba	13.10 ± 5.315Ba	15.72 ± 6.178Ba	0.4126
	p-value	<0.001**	<0.001**	0.001**	
WBC	Control	8.910± 1.838Aa	9.267 ± 1.807Aa	9.438 ± 1.809Aa	0.7485
	Abortion	7.248± 1.736Ba	7.738 ± 2.706Ba	10.99 ± 2.123Ab	0.0067**
	p-value	0.01**	0.02*	0.2655	
RBC	Control	4.260± 0.7370Aa	4.091 ± 0.5040Aa	4.153 ± 0.6066Aa	0.6721
	Abortion	4.279± 0.4670Aa	4.417 ± 0.5814Ba	4.730 ± 1.087Aa	0.3366
	p-value	0.9305	0.0447*	0.3674	
GOT	Control	25.59 ± 13.80Aa	18.60 ± 9.330Aa	23.80 ± 12.88Aa	0.1539
	Abortion	19.30 ± 7.666Aa	24.75 ± 7.951Ba	25.68 ± 5.983Aa	0.0687
	p-value	0.1144	0.02*	0.7591	
GPT	Control	22.11 ± 9.286Aa	16.01 ± 7.436Aa	24.23 ± 12.09Aa	0.0441*
	Abortion	16.62 ± 7.628Aa	23.61 ± 8.575Ba	23.33 ± 7.607Aa	0.0304*
	p-value	0.0611	0.001**	0.8885	
Systolic Blood Pressure, mmHg	Control	119.2 ± 10.77Aa	121.0 ± 8.891Aa	120.0 ± 0.000Aa	0.8287
	Abortion	120.0 ± 10.00Aa	121.0 ± 6.732Aa	118.3 ± 7.528Aa	0.7306
	p-value	0.6759	0.9705	0.6759	
Diastolic Blood Pressure, mmHg	Control	65.60 ± 7.118Aa	66.19 ± 7.400Aa	67.50 ± 5.000Aa	0.8736
	Abortion	70.67 ± 10.33Aa	72.14 ± 9.567Ba	73.33 ± 5.164Aa	0.8151
	p-value	0.0740	0.02*	0.1145	

* = significance under (p<0.05)

The result in (table 4) showed significantly differences in several biomarkers between the patients and control groups depending on genotype.

1. Vitamin D3 (VD3):

Highly significant differences were observed in the patients compared with control groups (CC, CT, TT), (12.42 ± 4.249 versus 36.02 ± 4.807, p= 0.001; 13.10 ± 5.3 versus 32.47 ± 3.302, p=0.001; 15.72 ± 6.178 versus 38.30 ± 6.487, p=0.001, respectively).This suggests a strong association between vitamin D3 deficiency and miscarriage.

2. White Blood Cells (WBC):

Significant differences were found in the patients compared with control groups (CC, CT) genotypes, (7.248 ± 1.736 versus 8.910 ± 1.838, p= 0.01; 7.738 ± 2.706 versus 9.267 ± 1.807, p= 0.02, respectively). No significant difference was observed in the TT genotype (p = 0.2675).

3. Red Blood Cells (RBC):

Significant difference was noted only in the CT genotype in the patients compared with control groups (4.417 ± 0.5814 versus 4.091 ± 0.5040, p = 0.0447), suggesting potential alterations in oxygen-carrying capacity in this subgroup.

4. GOT (AST – Aspartate Aminotransferase):

Significant elevation was detected solely in the CT genotype genotype, in the patients compared with control groups (24.75 ± 7.951versus 18.60 ± 9.330, p= 0.02), indicating possible hepatic stress or inflammatory activity related to miscarriage in this genotype.

5. GPT (ALT – Alanine Aminotransferase):

Significant differences were observed only in CT genotype, in the patients compared with control groups (23.61 ± 8.575 versus 16.01 ± 7.436, p=0.001) supporting the involvement of liver function alterations in miscarriage pathology for these genotypes.

Blood Pressure Parameters:

Systolic Blood Pressure:

No significant was observed in the genotype.

Diastolic Blood Pressure:

Also significantly different in the CT genotype, in the patients compared with control groups (72.14 ± 9.567 versus 66.19 ± 7.400, p= 0.02), reinforcing the link between this genotype and hemodynamic changes.

DISCUSSION

In this study, the association between the rs1544410 polymorphism in the Vitamin D Receptor (VDR) gene and the risk of recurrent miscarriage (RM) was evaluated in Iraqi women. Our results demonstrated a significant association between the dominant model (CT + TT vs. CC) and increased risk of miscarriage (OR = 2.33, 95% CI: 1.00–5.36, p = 0.04). These findings suggest that the C allele may act as a genetic susceptibility protector from miscarriage incidence.

This result aligns with previous reports that showed polymorphisms in the VDR gene, including rs1544410 (BsmI), are associated with various pregnancy-related complications such as gestational diabetes and preeclampsia [12, 13]. The reduced frequency of the protective CC genotype in patients (30% vs. 50% in controls) further supports a potential role of VDR polymorphisms in miscarriage pathology.

Importantly, the current study also revealed markedly reduced serum levels of vitamin D3 in all genotypes among the patient group compared to controls (p = 0.001 for all). Vitamin D is known to regulate immune tolerance during pregnancy, promote decidualization, and modulate trophoblast invasion [14, 15]. Deficiency in vitamin D may impair these processes, particularly in individuals with less efficient VDR signaling due to polymorphic variation, thereby increasing miscarriage risk.

In terms of hematological parameters, significant reductions in white blood cell counts (WBCs) were observed in patients with CC and CT genotypes, suggesting a possible dysregulated immune status. Moreover, the CT genotype was associated with reduced RBC counts, indicating altered oxygen delivery capacity, which may contribute to adverse pregnancy outcomes [16]. Regarding liver function, significant elevations in AST (GOT) and ALT (GPT) were detected in the CT genotype of the patient group. These enzymes are markers of hepatic inflammation, and their increase may reflect systemic or hepatic stress in women with recurrent miscarriage [17]. Additionally, a significant increase in diastolic blood pressure in CT genotype patients suggests vascular or hemodynamic instability, potentially compromising placental blood flow and leading to pregnancy failure [18].

Although the association was statistically significant only under the dominant model, the biological trends observed across hematological, biochemical, and cardiovascular indicators reinforce the hypothesis that VDR gene variants, combined with vitamin D deficiency and systemic inflammation, contribute to the multifactorial etiology of recurrent miscarriage.

Further studies with larger sample sizes and functional assays are required to clarify the mechanisms by which VDR polymorphisms influence pregnancy outcomes and to explore the potential benefit of vitamin D supplementation in genetically susceptible individuals.

CONCLUSION

This study demonstrates a potential association between specific polymorphisms in the VDR gene and the risk of recurrent pregnancy loss among Iraqi women.

The presence of certain rs1544410 of VDR gene may contribute to genetic susceptibility to miscarriage, possibly through disrupted vitamin D signaling pathways involved in immune modulation and placental function.

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