Association between Vitamin D Receptor Gene Foki Polymorphism and Gestational Diabetes Mellitus among Iraqi Pregnant Women

Raya Hatem Al-Mawla[1], Hameed Hussein Ali[2], Wajeeh Younis Al-Ani[2]

[1]Research Scholar, [2]Assist Prof. Dr. Department of chemistry, college of Science, University of Anbar, Ramadi, Iraq

Abstract

This study was conducted to evaluate the association between vitamin D receptor gene FokI polymorphism and gestational diabetes Mellitus (GDM) among Iraqi pregnant women.

This study included 40 women with GDM, 30 pregnant women without GDM, and 30 non-pregnant women as a negative control. Age (years), body mass index (BMI), Fasting Blood Glucose (FBG), total cholesterol, triglycerides (TG), and lipid profile of the patients and control groups were compared (Table 1). DNA sampling was purified and amplified by using PCR. The FokI genotype was evaluated using restriction fragment length polymorphism (RFLP).

The results revealed that the age, BMI, FBG, HbAIc, insulin hormone, insulin resistance, and lipid profile except HDL-C were significantly increased with an increased GDM risk compared to women with normal pregnancies. In contrast, no significant alterations were observed in concentrations of IGF1 hormone, vitamin D and FokI (F and f) alleles frequency between GDM and control groups.

This study suggests that there was no significant correlation between the VDR FokI polymorphism and GDM patients among Iraqi pregnant women.

Keywords: *Gestational diabetes, FokI, Polymorphism.*

Introduction

Vitamin D is secosteroid produced in the skin due to the effect of solar ultraviolet B radiation. Two consecutive hydroxylation in the liver and kidney leads to form a functional 1, 25 –dihydroxy vitamin D [(1)]. The two most important forms of vitamin D are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) which cannot produce in the human body and consume with fortified food or given by supplements [(2)]. These two form are bound to the vitamin D binding protein in human plasma and transported them to the liver where both are hydroxylated to form 25-hydoxy vitamin D [(3)].

Vitamin D has an influence on the expression of over 200 different genes. Deficiency maybe related to diabetes [(4),] different forms of cancer, cardiovascular disease, autoimmune diseases and innate immunity. [(2)]

Correlation between the (VDR) gene and diabetes has been noted in several population. Which is encoded by a gene located in chromosome 12q12 and several polymorphisms were used for its description.[(5)] In addition, this gene is presented in the pancreatic β-cells. Consequently,1,25$(OH)_2D_3$ may has a role in insulin secretion and sensitivity in diabetes by either increasing the intracellular calcium concentration in the β-cell to induce insulin secretion or by increasing the conversion of pro insulin to insulin. [(6)] VDR gene FokI (RFLP) in exon 2 [(7)]

Raya Hatem Al-Mawla
E-mail: athmawla@yahoo.com

Gestational diabetes mellitus (GDM) usually reveals itself in the latter half of pregnancy and it is identified by carbohydrate intolerance of variable severity. [8] Hyperglycemia development during pregnancy may lead to insulin resistance due to secretion of placental hormones [9]

Several factors are led to increase GDM such as glucose urea, age over 30 years, obesity, family history of diabetes, a previous history of GDM and macrosomic child.[10] Vitamin D deficiency may play a role in the pathogenesis of GDM [11]

The present study aimed to investigate association between VDR-FokI gene polymorphism and risk of GDM among Iraqi pregnant women.

Materials and Methods

A total of 100 blood samples were collected from 40 pregnant GDM, 30 pregnant without GDM and 30 healthy non-pregnant Iraqi women after 10-12 hours fasting (control group). Age (years), body mass index – BMI (Kg/m^2) were recorded. The blood sample was divided into two aliquots, the first (3 ml) of separated serum used for assays of FBG (mg/dl), total cholesterol (mg/dl), TG (mg/dl), HDL-C (mg/dl), LDL– C (mg /dl), VLDL – C (mg /dl), Insulin hormone (MIU/ml), IGF1(ng/ml) and vitamin D (ng / ml). the second aliquots (2 ml) was collected in EDTA tube for HbA1c determination and then used to DNA extraction. DNA was extracted using genomic lysis buffer and was checked for purity and concentration. DNA samples were all quoted and stored at

PCR mixture was carried out with in the presence of DNA (1.5 µl), 5µl Taq PCR premix (5 U/µl Taq DNA polymerase, 2.5 mM DNTPs,1µl 10x reaction buffer and 1 µl gel loading buffer), 10 picomoles/µl of each primer (table 1) and 16.5 µl distill water.

PCR thermal cycler was programmed as follows: denaturation step at 95 ˚C for 3 minutes followed by 35 cycles at 95˚C for 45 seconds and followed annealing by 35 cycles at 68˚C for 45 seconds and extension by 35 cycles at 72 ˚C for 45 seconds, final extension cycle at 72˚C for 7 minutes was done. PCR products were analyzed on 1.5 % agarose gel and UV transmission.

Restriction fragment length polymorphism (RFLP-PCR) was used to identify VDR genotypes. PCR product (5µl) was digested (37ºC for 30minutes) with 0.5 µl Fok I restriction enzyme. Digested product was electrophoresed on 2.5% agarose gel. Genotype was determined according to fragments length i.e. homozygote (FF) subjects = 270bp product ; heterozygote (Ff) subjects = 270,210 and 60 bp products and homozygote (ff) subjects = 110,60 bp products. SNP resulting in T-C substitution in exon 2 of VDR gene leads to the generation of a FokI restriction site. Homozygous subjects with alleles containing nucleotide T at this position showed two bands of 210 and 60 bp and were designated as having ff FokI genotype. Homozygous subjects with alleles containing C at this position showed an intact 270 bp band (FF) subjects. Heterozygote subjects showed all 3 bands: 270,210 and 60bp and were designated Ff.

Table 1: Primer used for polymerase chain reaction (PCR) of VDR FokI gene.

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5-AGTGGCCCTGGCACTGACTCTGGCTCT-3	68.6	63	270 Base pair
Reverse	5-ATGGAAACACCTTGCTTCTTCTCCCCTC-3	60.5	48.1	

VDR: Vitamin D receptor.

Results

Total number of 40 GDM pregnant, 30 healthy pregnant women and 30 controls without pregnant and have normal concentration of vitamin D were recruited in our study. Age, BMI, FBG, HbA1c, Insulin hormone, insulin resistance and lipid profile except HDL-C were statistically significant difference between normal pregnant and gestational diabetes mellitus ($p \leq 0.05$), but there were no significant differences in concentrations in IGF1 hormone and vitamin D ($p > 0.05$) (Table 2).

Table 2: Demographic and biochemical data of GDM and controls.

Parameter	GDM	Normal pregnant	control	P value
Age (Years)	35.175	27.500	30.933	P LSD=2.95< 0.001
BMI (Kg/m2)	30.723	26.569	24.196	PLSD=1.68< 0.001
FBG (mg/dl)	145.950	89.400	86.200	PLSD=11.6< 0.001
HbA1c (%)	7.082	5.046	4.840	PLSD=0.539< 0.001
Ins H (MIU/ml)	14.782	9.293	10.707	PLSD=3.346<0.003
HOMA-IR (%)	4.912	2.048	2.245	PLSD=0.956<0.001
Vitamin D (ng/ml)	6.833	8.733	23.809	PLSD=2.161<0.001
IGF1 (ng/ml)	99.350	90.517	63.100	PLSD=15.86<0.001
TG (mg/dl)	194.550	164.567	133.500	PLSD=16.30<0.001
Cholesterol (mg/dl)	225.17	175.33	179.37	PLSD=23.38<0.001
HDL-C (mg/dl)	38.000	35.567	41.833	PLSD=3.820<0.009
LDL (mg/dl)	146.78	107.53	111.06	PLSD=22.76<0,001
VLDL (mg/dl)	38.910	32.900	26.370	PLSD=3.224<0.001

Figure 1 shows PCR product of 270 bp for VDR FokI gene.

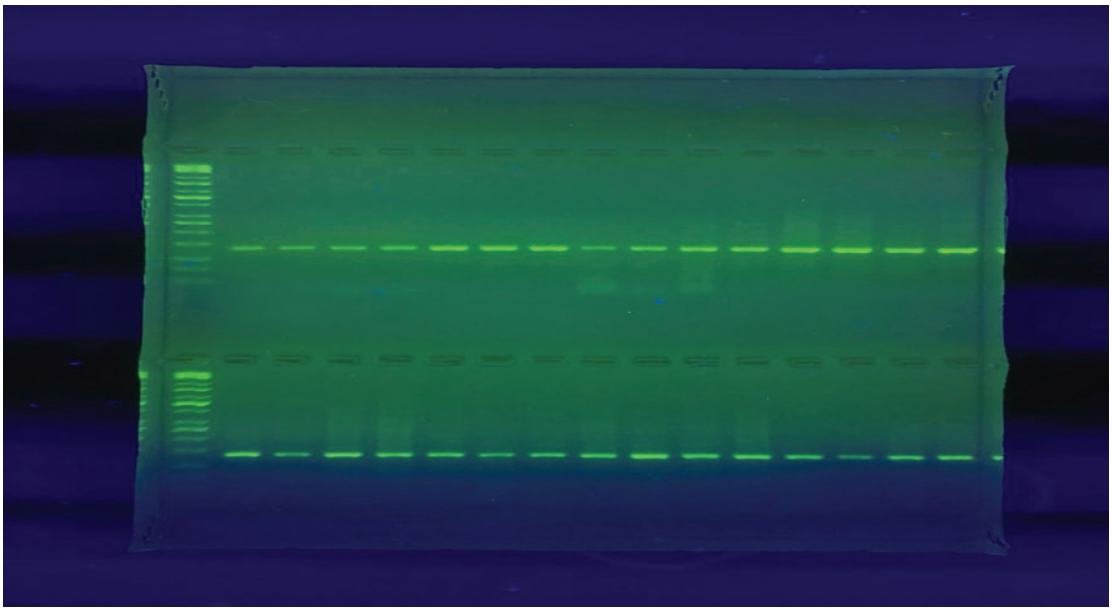

Figure 1: 1.5% agarose gel electrophoresis of VDR PCR for SNP BsmI enzyme showing a wild type FF 270 bp product.

Genotype of VDR FokI gene polymorphism determined according to the fragments length, homozygote FF

remained undigested in 270bp, homozygote ff was digested to 210 and 60 bp and heterozygote Ff digested to 270,210 and 60 bp.These results show insignificant differences in GDM patients and controls (Table 3).

Table 3: Genotype of Fok1 polymorphism.

Gene	**Genotype**	**Gestational No. (%)**	**Control samples No. (%)**	**OR (95% CI)**	**P value**
VDR FOK1	FF	(45.45%)	(60.00%)	0.56 (0.18 -1.73)	0.241
	Ff	(54.55%)	(36.00%)	2.13 (0.68 -6.71)	0.163
	ff	(0.00%)	(4.00%)	0.36 (0.02 -8.75)	0.532
Gene	Genotype	GDM No. (%)	Control samples No. (%)	OR (95% CI)	P value
VDR FOK1	FF	(54.17%)	(60.00%)	0.79 (0.26 -2.39)	0.451
	Ff	(37.50%)	(36.00%)	1.07 (0.34 -3.33)	0.574
	ff	(8.33%)	(4.00%)	2.18 (0.19-24.5)	0.484
Gene	Genotype	Gestational No. (%)	GDM No. (%)	OR (95% CI)	P value
VDR FOK1	FF	(45.45%)	(54.17%)	0.71 (0.23 -2.20)	0.812
	Ff	(54.55%)	(37.50%)	2.00 (0.63 -6.33)	0.928
	ff	(0.00%)	(8.33%)	0.20 (0.01 -4.12)	1.000

Allele frequency of VDR FokI gene polymorphism in GDM patients and controls show in table 4.

Table 4: Allele frequency of Fok1 polymorphism.

Gene	Allele	Gestational No. (%)	Control samples No. (%)	OR (95% CI)	P value
VDR FOK1	F	(72.73%)	(78.0%)	0.75 (0.30- 1.91)	0.361
	f	(27.27%)	(22.0%)	1.33 (0.52 - 3.38)	0.361
Gene	Allele	GDM No. (%)	Control samples No. (%)	OR (95% CI)	P value
VDR FOK1	F	(72.92%)	(78.0%)	0.76 (0.30 - 1.89)	0.363
	f	(27.08%)	(22.0%)	1.32 (0.53 - 3.29)	0.363
Gene	Allele	Gestational No. (%)	GDM No. (%)	OR (95% CI)	P value
VDR FOK1	F	(72.73%)	(72.92%)	0.99 (0.40 - 2.46)	0.601
	f	(27.27%)	(27.08%)	1.01 (0.41 - 2.51)	0.601

Discussion

Diabetes mellitus became major health concern worldwide. Female < 50 years (gestational age) have greater prevalence than males [12]. VDR polymorphism influences capability to type 1 diabetes mellitus, but association with GDM is not yet clear.[13] Vitamin D has indicated to be substantially related to pancreatic β-cell function and susceptibility [14]. It was advertised that, no association noticed between VDR BsmI polymorphism and GDM in Saudi women [15]

We analyzed VDR gene FokI polymorphism in GDM pregnant Iraqi women. Genotypes frequencies for FF, Ff, ff among control groups were 60%, 36% and 4% respectively, and among normal pregnant were 45.45%, 54.55% and 0.0% respectively, with insignificant association ($p > 0.05$), and in GDM patients were 54.17%, 37.50% and 8.33% respectively with no significance with normal pregnant and with control ($p > 0.05$). These finding are in agreement with results of the study that showed VDR FokI polymorphism not associated with Saudi GDM [13]. An Indian study suggested that VDR gene polymorphism is associated with type 2 diabetes mellitus.[16] Results of allele frequency distribution F and f in GDM were 72.92% and 27.08% respectively, in control were 78.0 % and 22.0 %, and among normal pregnant were 72.73 % and 27.27%, and there were no significantly between these groups ($P > 0.05$). These results consistent with got by Aslani S and Alzaim M, who suggested that F allele may have a role in decrease incidence of GDM [17]

Conclusion

Vitamin D deficiency was more prevalent among Iraqi women. The results showed a relationship between age, body mass index and gestational diabetes mellitus, also There were significant differences in concentrations of fasting blood glucose, glycated hemoglobin, insulin hormone, insulin resistance, lipid profile except high density lipoprotein cholesterol between normal pregnant and gestational diabetes mellitus, but there were no significant differences in concentrations in insulin – like growth factor hormone.The results of the present study showed vitamin D receptor FokI polymorphisms

not associated with Iraqi gestational dibetes mellitus. Homozygous FF turned out to be as a prenetive genotype of gestational diabetes mellitus,while Ff and ff turned out to be related with risk of GDM.The allele F represents a preventive allele, while the allele f acts as etiological factor.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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