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ASSOCIATION OF VITAMIN D_3 RECEPTOR GENE (BSMI) AND SOME BIOCHEMICAL PARAMETERS IN IRAQI GESTATIONAL DIABETIC PATIENTS

Raya Hatem Al-Mawla*, Hameed Hussein Ali and Wajeeh Younis Al-Ani

Department of Chemistry, College of Science, University of Anbar, Ramadi, Iraq.
*e-mail: athmawla@yahoo.com

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ABSTRACT : Our study was conducted to estimate the association between vitamin D_3 receptor gene (BsmI) polymorphism and gestational diabetes mellitus (GDM) patients among Iraqi women. This study involved 40 women with GDM, 30 normal pregnant women as a positive control 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 studying groups were determined. DNA sampling was purified and amplified by using PCR. The BsmI genotype was estimated using Restriction Fragment Length Polymorphism (RFLP). The results exposed that the age, BMI, FBG, HbAIc, insulin hormone, insulin resistance, and lipid profile except HDL-C were significantly increased with GDM risk compared to women with normal pregnancies, but there was no significant alterations in concentrations of IGF1 hormone, vitamin D and BsmI (B and b) alleles frequency between GDM and control groups. This study offers that there was no significant correlation between BsmI polymorphism and GDM patients among Iraqi pregnant women.

Key words : Gestational diabetes, BsmI, polymorphism.

INTRODUCTION

Vitamin D is asecosteroid hormone may be produced in the skin because of the effect of solar ultraviolet B radiation. Two successive hydroxylation in the liver and the kidney leads to form a functional 1,25 –dihydroxy vitamin D (Holick, 2002). The important forms of vitamin D are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). These two form are bound to the vitamin D binding protein in plasma and transferred them to hydroxylated in the liver to form 25-hydoxy vitamin D (Houghton *et al*, 2006).

Vitamin D has an effect on the expressionmore than 200 different genes. Deficiency maybe related to diabetes (IDE, 2014).

Relationship between the (VDR) gene and diabetes may be noted in several population. Which is encoded by a gene located in chromosome 12q12 and several polymorphisms were used for its description (Zmuda *et al*, 2000). In addition, this gene is presentedin the pancreatic â-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 â-cell to induce insulin secretion or by increasing the conversion of pro insulin to insulin (Seshadri *et al*, 2011) VDR gene BsmI located in intron 8 (Pittas *et al*, 2007).

Gestational diabetes mellitus (GDM) usually happens in the latter half of pregnancy and it is determined by carbohydrate intolerance (Shukla *et al*, 2015). Hyperglycemia development during pregnancy may lead to insulin resistance due to secretion of placental hormones (ADA, 2003).

Vitamin D_3 deficiency may play a role in the pathogenesis of GDM (Bodnar *et al*, 2011).

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

MATERIALS AND METHODS

One hundred blood samples were collected from 40 pregnantwith GDM, 30 normal pregnant and 30 healthy nonpregnant Iraqi women after 10 -12 hours fasting as 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 /

Table 1 : Primer used for polymerase chain reaction (PCR) of VDRBsmI gene (El-Beshbishy *et al*, 2015).

Primer	Sequence	Tm(^{0}C)	GC(%)	Product size
Forward	5- CAACCAAGACTACAAGTACCGCGTCAGTGA-3	62.8	50	820base pair
Reverse	5- AACCAGCGGGAAGAGGTCAAGGG-3	63.3	60.9	

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.

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, (1 µl) gel loading buffer, (10 picomoles/µl) of primer (table 1) and 16.5 µl distill water.

PCR thermal cycler was programmed as follows: denaturation step at 95^{0}C for 3 minutes followed by 35 cycles at 95^{0}C for 45 seconds and followed annealing by 35 cycles at 68^{0}C for 45 seconds and extension by 35 cycles at 72^{0}C for 45 seconds, final extension cycle at 72^{0}C for 7 minutes was done.

Restriction fragment length polymorphism (RFLP-PCR) was used to identify VDR genotypes.PCR product (5 µl) was digested at 37^{0}C for 30 minutes with 0.5 µl BsmI restriction enzyme. Digested product electrophoretically seperated on a 3% agarose gel stained with Red Safe.Genotypewas determined according to fragments length *i.e.* homozygote (BB) subjects =820 bp product; heterozygote (bb) subjects = 820, 650 and 170 bp products.

RESULTS

Cases of GDM pregnant, healthy pregnant and 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).

Moleculer analysis

The purity of the nucleic acid were detected by nanodrop UV spectrophotometer. The amplified fragment with a size 820 bp could be viewed in the 1.5% agarose gel after electrophoresis at 5 volt/cm^2.

Genotype of VDR BsmI gene polymorphism determined to the fragments length, Homozygote BB genotype remained undigested in 820bp. Homozygote bb genotype was digested to three band 820, 650 and 170 bp. There was not band for genotype Bb in 650 and 170 bp in our studying as shown in Fig. 2.

Results of allele frequency of VDR BsmI gene polymorphism in GDMpatients and controls show in Table 4.

DISCUSSION

Diabetes mellitus became major health concern worldwide. Female < 50 years have greater prevalence than males (Alqurashi *et al*, 2011). VDR polymorphism influences capability to diabetes mellitus, but association with GDM is not yet clear (El-Beshbishy *et al*, 2015). Vitamin D has related to pancreatic â-cell action and sensibility (Ortlepp *et al*, 2003). No related perceived between VDR BsmI polymorphism and GDM in Saudi

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/m^2)	30.723	26.569	24.196	$P_{LSD=1.68} < 0.001$
FBG (mg/dl)	145.950	89.400	86.200	$P_{LSD=11.6} < 0.001$
HbA1c (%)	7.082	5.046	4.840	$P_{LSD=0.539} < 0.001$
Ins H (MIU/ml)	14.782	9.293	10.707	$P_{LSD=3.346} < 0.003$
HOMA-IR (%)	4.912	2.048	2.245	$P_{LSD=0.956} < 0.001$
Vitamin D (ng/ml)	6.833	8.733	23.809	$P_{LSD=2.161} < 0.001$
IGF1 (ng/ml)	99.350	90.517	63.100	$P_{LSD=15.86} < 0.001$
TG (mg/dl)	194.550	164.567	133.500	$P_{LSD=16.30} < 0.001$
Cholesterol (mg/dl)	225.17	175.33	179.37	$P_{LSD=23.38} < 0.001$
HDL-C (mg/dl)	38.000	35.567	41.833	$P_{LSD=3.820} < 0.009$
LDL (mg/dl)	146.78	107.53	111.06	$P_{LSD=22.76} < 0,001$
VLDL (mg/dl)	38.910	32.900	26.370	$P_{LSD=3.224} < 0.001$

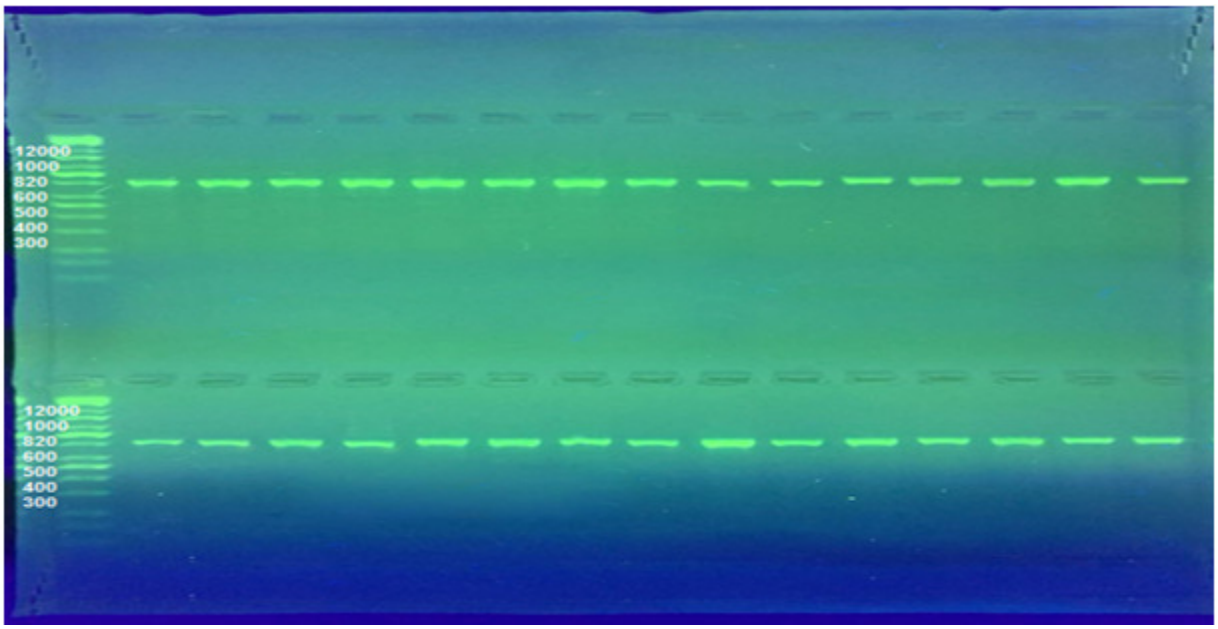

Fig. 1 : PCR product of the VDR gene BsmI at the band size 820 bp, at 1.5 % agarose gel at 5 volt/cm^2.

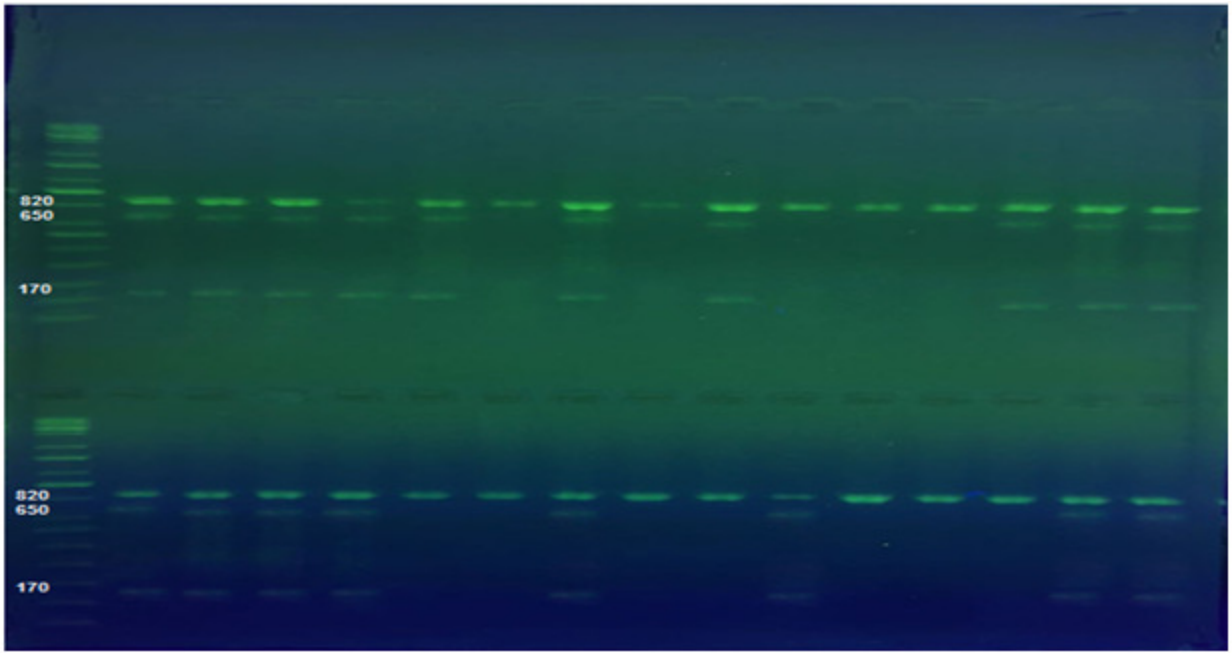

Fig. 2 : Electrophoresis pattern of PCR product digested with BsmI restriction enzyme.

women (Tawfeek *et al*, 2011).

We analyzed VDR gene BsmI polymorphism in GDM pregnant Iraqi women. Genotypes frequencies forBB, Bb, bb among control groups were 44%, 56% and 0.0% respectively and among normal pregnant were 36%, 63% and 0.0%, respectively, with insignificant association ($p > 0.05$) and in GDM patients were 37%, 62% and 0.0% respectively with no significance with normal pregnant and with control ($p > 0.05$). Results obtained by different investigators who studied BsmIpolymorphism varied among diabetics, BsmI polymorphism has been linked to susceptibility to diabetes inseveral countries (Motohashi *et al*, 2003; Ban, 2001). Other studies in other countries could not establish association between BsmI and existence of diabetes (El-Beshbishy *et al*, 2015; Turpeinen *et al*, 2003; Angel *et al*, 2003).

Results elucidated variation in genotyping frequency between studying groups. BB genotype in healthy control was higher than patients with GDM, but no significant difference was observed, where odds ratio was equal to 0.76 with confidence intervals 95% CI (0.25-2.34), which represents preventive factor from GDM. While ratio of Bb pattern appears as genotype related with risk of GDM (OR = 1.31) with confidence intervals 95% (0.43 - 4.01).

Table 3 : Genotype of Bsm1 polymorphism.

Gene	Genotype	Gestational No.(%)	Control No.(%)	OR (95% CI)	*P* -value
*VDR*BsmI	BB	11(36.36%)	13 (44.00%)	0.73 (0.23 -2.29)	0.408
	Bb	19(63.64%)	17 (56.00%)	1.37 (0.44 - 4.34)	0.408
	bb	0 (0.00%)	0 (0.00%)	1.00 (0.07 - 18.27)	1.000
Gene	**Genotype**	**GDM No. (%)**	**Control No.(%)**	**OR (95% CI)**	***P* -value**
*VDR*BsmI	BB	15 (37.50%)	13(44.00%)	0.76 (0.25 - 2.34)	0.432
	Bb	25(62.50%)	17 (56.00%)	1.31 (0.43 - 4.01)	0.432
	bb	0 (0.00%)	0(0.00%)	(0.07 - 18.27)	1.000
Gene	**Genotype**	**Gestational No.(%)**	**GDM No.(%)**	**OR (95% CI)**	***P* value**
*VDR*BsmI	BB	11(36.36%)	15 (37.50%)	0.95 (0.29 - 3.08)	0.649
	Bb	19(63.64%)	25 (62.50%)	1.05 (0.33 - 3.39)	0.649
	bb	0 (0.00%)	0 (0.00%)	1.00 (0.07 - 18.27)	1.000

Table 4 : Allele frequency of Bsm1 polymorphism.

Gene	Allele	Gestational No (%)	Control No. (%)	OR(95% CI)	*P* value
VDRBsmI	B	20(68.18%)	22 (78.0%)	0.83 (0.35 - 2.00)	0.429
	b	10(31.82%)	8 (22.0%)	1.33 (0.52 - 3.38)	0.361
Gene	**Allele**	**GDM No. (%)**	**Control No. (%)**	**OR(95% CI)**	***P* value**
VDRBsmI	B	28(68.75%)	22(78.0%)	0.76 (0.30 - 1.89)	0.363
	b	12(31.25%)	8(22.0%)	1.06 (0.44 - 2.52)	0.538
Gene	**Allele**	**Gestational No. (%)**	**GDMNo.(%)**	**OR(95% CI)**	***P* value**
VDRBsmI	B	20(68.18%)	28(68.75%)	0.97 (0.41 - 2.33)	0.612
	b	10(31.82%)	12(31.25%)	1.03(0.43 - 2.45)	0.612

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 BsmI polymorphisms not associated with Iraqi gestational dibetes mellitus. Homozygous BB turned out to be as a prenetive genotype of gestational diabetes mellitus, while Bb turned out to be related with risk of GDM. The allele B represents a preventive allele, while the allele b acts as etiological factor.

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