

Association of Vitamin D Receptor Gene Polymorphism with Metabolic Syndrome and Type 2 Diabetes Mellitus in a Sample of Egyptian Patients

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ABSTRACT

Background. There are insufficient data on the association of vitamin D receptor (VDR) genes polymorphism and type 2 diabetes mellitus (type 2 DM), and various components of metabolic syndrome among Egyptian patients. The aim of the present study was to study the association of different SNPs of VDR genes BsmI, ApaI, TaqI and FokI and components of metabolic syndrome and type 2 DM among cohort of Egyptian patients. **Methods.** The study is a case-control study. Patients included in the study were divided into three groups. Group 1 included 78 patients with type 2 DM; group 2 included 72 patients with metabolic syndrome and one hundred age-matched healthy subjects were served as control group. Full biochemical study and serum 25-hydroxy vitamin D (25(OH)D) were done. Purified DNA was subjected to study with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) for genotyping of SNPs of VDR gene. Data were presented as mean and standard deviation, and were analysed as appropriate by using the one-way ANOVA or paired *t*-test. Pearson correlation coefficient was used to correlate between variables. **Results.** Study of VDR genetic polymorphism had shown significant increase in the prevalence of Ff genotypes among diabetic patients and patients with manifestations of metabolic syndrome. There was significant negative correlation between 25(OH)D and total cholesterol, triglyceride, fasting and post-prandial blood glucose levels, waist circumference and diastolic blood pressure. **Conclusion.** The genetic polymorphism of VDR might play a role in the pathophysiology of type 2 DM and metabolic syndrome., however, more longitudinal studies are still required to support these finding.

Key words: Metabolic syndrome, Diabetes mellitus, Polymorphism, Vitamin D receptor.

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INTRODUCTION

Diabetes mellitus (DM) is a global non communicable disease. It is estimated to affect around 285 million individuals worldwide [1]. This disorder is associated with various factors that may have a pathogenic role in its development. One of these factors is the deficiency of vitamin D [2,3]. Various clinical studies have reported a positive correlation between

the level of the circulating 25-hydroxy vitamin D (25(OH)D) and insulin sensitivity, thus the deficiency of vitamin D may predispose to the altered insulin sensitivity, hyperglycemia and type 2 DM [4]. There is also evidence that vitamin D deficiency may be associated with the manifestations of metabolic syndrome, namely dyslipidemia, hyperglycemia, hypertension and obesity [5]. Likewise, the association of reduced levels of vitamin D and the risk factors leading

to cardiac diseases may denote the potential extra skeletal functions of this sterol hormone [6, 7]. The association between obesity and increased adipose tissues is thought to be the key factor for all the previous disorders. This is thought to be mediated, at least partially, through the reducing effect of adipose tissue on vitamin D level by increased expression of vitamin D metabolizing enzymes such as 25-hydroxylase CYP2J2, CYP27B1, and CYP24 [8-10].

The mechanism of vitamin D function includes binding of its active metabolite 1,25-hydroxy vitamin D with vitamin D receptor (VDR) [11]. The VDR receptor belongs to steroid/thyroid hormone family of receptors and it functions as a transcriptional activator of many genes [12]. Single nucleotide polymorphisms (SNPs) in the VDR gene can affect the activity of the VDR that have been associated with various metabolic disorders [13-15]. VDR gene is located on chromosome 12q and there is more than 470 reported SNPs that can be considered as candidates for various disease risks [16]. An important VDR SNP includes the rs10735810/rs2228570 (FokI) situated in exon 2. There are also three SNPs in linkage disequilibrium, namely rs1544410 (BsmI) located in intron 8, rs731236 (TaqI), and rs7975232 (ApaI), the last being a SNP located in exon 9 and intron 9 [17, 18]. Since there are insufficient data on the association of VDR gene polymorphism with type 2 DM and various components of metabolic syndrome among Egyptian patients, therefore the aim of this piece of research was to investigate the association of different SNPs of VDR genes, namely FokI, BsmI, ApaI, and TaqI with different components of metabolic syndrome and type 2 DM among a cohort of Egyptian patients.

MATERIALS AND METHODS

Study design

The study was conducted at Mansoura University Hospital from July 2017 till July 2018. The study is a case –control study. Subjects included in the study were divided into three groups. Group 1 included patients with type 2 DM as defined by the World Health Organization criteria having fasting blood glucose level 126 mg/dl or more, and/or 2h postprandial blood glucose level 200 mg/dl or more [19]. Exclusion criteria included: the presence of chronic illnesses that potentially alter vitamin D metabolism, pregnant or breastfeeding women, and the use of any variant of vitamin D supplements. The second group included patients with

evidence of metabolic syndrome with minimum 3 of 5 of the following criteria: waist circumference equals 102 cm or more in men, and 88 cm or more in women; elevated fasting blood glucose above 110 mg/dl; elevated triglycerides above 150 mg/dl; reduced HDL-cholesterol below 40 g/dl for men and below 50 mg/dl for women and elevated systolic blood pressure above 130 mmHg or diastolic blood pressure above 85 mmHg [20]. In addition, one hundred healthy subjects with age match were enrolled as control group. All included subjects were also evaluated by clinical examinations. The study was approved by Mansoura University Faculty of Medicine Ethical Committee and was conducted according to the regulations of Declaration of Helsinki regarding conducting clinical research on human subjects. An informed written consent was obtained from each participant.

Biochemical studies

From each subject 10 ml blood sample was withdrawn and divided into two aliquots: one with EDTA and the other was left plain. From the plain tubes, sera were separated and subjected to full biochemical study of total triglycerides, total cholesterol, HDL-cholesterol, fasting and post prandial blood glucose levels using commercially available assay kits. Total calcium was measured by Dialab 450 autoanalyzer and ionized calcium by GEM premier 3500 analyzer (Boston, SN:10090790). The total serum 25(OH)D was measured by ELISA kits (Calbiotech, CA, USA) according to the manufacturer's instructions.

Genotyping of VDR polymorphism

Blood samples on EDTA were subjected to leucocytes isolation by Ficoll gradient method (Sigma-Aldrich, St. Louis, MO, USA). DNA was extracted by the use of DNA mini extract kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The presence of the VDR FokI (rs2228570), BsmI (rs1544410), ApaI (rs7975232) and TaqI (rs731236) SNPs was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the manufacturer's instructions (New England BioLabs, Ipswich, USA). Restriction fragment size analysis was performed by visualization of digested PCR product by 2% agarose gel electrophoresis and ethidium bromide staining. Primer sequences and conditions for PCR-RFLP analyses are presented in Table 1. The presence

Table 1. Genetic polymorphisms primers, restriction endonuclease enzymes and sequences

| | | | |
|-------------------|--|-----------------------------------|--|
| FokI polymorphism | Forward: 5' - AGCTGGCCCTGGCACTGACTCTG-GCTCT-3' Reverse: 5' - ATGGAAACACCTTGCTTCTTCTC-CCTC -3' | FokI restriction enzyme | The wild type homozygote FF-270 bp heterozygote (Ff-270, 210 and 60 bp) and mutant homozygote (ff-210 and 60 bp) |
| BsmI polymorphism | Forward: 5' - CAACCAAGACTACAAGTACCGCGT-CAGTGA-3' Reverse: 5' -AACCAGCGGGAAGAGGTCAAGGG-3' | BsmI restriction enzyme | The wild type homozygote (BB-820 bp), heterozygote (Bb-820, 650 and 170 bp) and mutant homozygote (bb-650 and 170 bp) |
| ApaI polymorphism | Forward: 5' - CAGAGCATGGACAGG GAGCAA-3' Reverse: 5' - GCAACTCCTCATGGCTGAGGTCTC-3' | ApaI restriction enzyme (Promega) | Absence of ApaI restriction site (2000bp) was assigned as a common allele A (wild-type allele) and presence of restriction site resulting in 1700 bp and 300bp fragments was assigned as infrequent allele a (mutant allele) Presence of 2000, 1700 and 300 bp fragments was assigned as heterozygotes (Aa). |
| TaqI polymorphism | (S'-CAGAGCATGGACAGGGAG-CAA-3') and a reverse primer in exon 9 (S'-GCAACTCCTCATGGCT GAG-GTCTC-3') | TaqI restriction endonuclease | Homozygous absence of the TaqI polymorphism results in two fragments of 245 bp and 495 bp, while homozygous presence of the site results in three fragments of 290,245, and 205 bp. Heterozygotes exhibit fragments of 490, 290, 245, and 205 bp |

of the FokI, BsmI, ApaI and TaqI polymorphisms was confirmed by repeated PCR-RFLP analysis. The details of the amplification procedures were previously described [21, 22].

Statistical analysis

Statistical analysis was performed by SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as mean \pm standard deviation (SD). Multiple or pair-wise comparison between means was done as appropriate by using one-way ANOVA or the paired *t*-test. The chi-square was used for pair-wise comparison between genotypes proportions. Pearson's correlation coefficient was used to calculate correlations between variables. Statistical significance was assumed when P values are ≤ 0.05 .

RESULTS

The study included three groups, the first group included 78 diabetic patients with mean age 45.4 ± 8.9 years mainly females (57.7%). The second group included 72 patients with criteria indicating that they had metabolic syndrome with mean age 44.5 ± 6.9 years. They were mainly females (54.2%). The third group included 100 healthy control subjects with mean age 43.0 ± 5.6 years and equal gender distribution. Table 2 summarizes the demographic, clinical and laboratory findings of the studied groups. As shown in Table 2, there was statistically significant decrease in the levels of total calcium and 25(OH)D in diabetic patients and patients with metabolic syndrome as compared to healthy control subjects ($P \leq 0.001$). Additionally, there were significant elevated levels of total cholesterol, triglycerides, fasting blood glucose

Table 2. Demographic, clinical and laboratory findings of the studied groups

| | Control (n=100) | Diabetic patients (n=78) | Patients with metabolic syndrome (n=72) |
|---------------------------------|--------------------|-----------------------------|--|
| Gender | | | |
| Male | 50 (50%) | 33 (42.3%) | 33 (45.8%) |
| Female | 50 (50%) | 45 (57.7%) | 39 (54.2%) |
| Age | 43.0 ± 5.6 | 45.4 ± 8.4 | 44.5 ± 6.9 |
| Waist circumference (cm) | 91.8 ± 6.4 | 112.4 ± 5.5‡ | 90.1 ± 5.3* |
| Systolic blood pressure (mmHg) | 108.7 ± 3.79 | 116.9 ± 6.1‡ | 106.5 ± 4.15† |
| Diastolic blood pressure (mmHg) | 75.7 ± 4.1 | 85.6 ± 4.07‡ | 77.2 ± 3.13* |
| Total calcium (mg/dl) | 9.5 ± 0.42 | 8.2 ± 0.57‡ | 8.3 ± 0.64‡ |
| Ionized calcium (mg/dl) | 4.1 ± 0.17 | 4.0 ± 0.12† | 4.05 ± 0.17* |
| 25(OH)D (nmol/l) | 140.0 ± 4.1 | 71.8 ± 3.6‡ | 71.0 ± 4.1‡ |
| Total cholesterol (mg/dl) | 182.7 ± 6.7 | 265.5 ± 30.1‡ | 181.7 ± 11.0 |
| HDL-cholesterol (mg/dl) | 53.9 ± 1.5 | 40.8 ± 4.4‡ | 52.3 ± 2.4* |
| Triglycerides (mg/dl) | 122.9 ± 5.3 | 232.1 ± 47.3‡ | 173.0 ± 12.7‡ |
| Fasting blood glucose (mg/dl) | 103.0 ± 6.3 | 223.0 ± 38.6‡ | 125.2 ± 2.01‡ |
| Post prandial glucose (mg/dl) | 112.5 ± 5.3 | 275.3 ± 37.6‡ | 129.5 ± 2.5‡ |

Data are shown as mean ± SD. Significance levels: *P≤0.05, †P≤0.01, ‡P≤0.001 vs control group (Paired *t*-test).

and postprandial blood glucose levels in both two patients groups compared to healthy controls (P≤0.001).

Study of VDR genetic polymorphism had shown significant increase in the prevalence of Ff genotypes among diabetic patients and patients with manifestations of metabolic syndrome (P≤0.05, Table 3). In the study between 25(OH)D levels and various biochemical parameters there was significant negative correlation between 25(OH)D and total cholesterol, triglyceride, fasting and post prandial blood glucose levels (P≤0.001). Likewise, there was significant negative correlation between 25(OH)D and both waist circumference (and body-mass index [BMI], data not shown) and diastolic blood pressure (P≤0.001). On the contrary, there was significant positive correlation between 25(OH)D and total calcium level (P≤0.001, Table 4).

As shown in Table 5, the study of the association between FokI genotypes and clinical and laboratory findings among both diabetic patients and patients with with metabolic syndrome had revealed significant correlation between decreased calcium, HDL-cholesterol and 25(OH)D levels and Ff genotype (P≤0.001, P≤0.05, P≤0.05 respectively). There were also significant increase in the risk factors associated with metabolic syndrome such as total cholesterol

Table 3. Genetic polymorphism of VDR among the studied groups

| VDR Polymorphism | Control (n=100) | Diabetic patients (n=78) | Patients with metabolic syndrome (n=72) |
|------------------|--------------------|-----------------------------|---|
| BsmI | | | |
| BB | 20 (20%) | 33 (42.3%)† | 27 (37.5%)* |
| Bb | 30 (30%) | 39 (50%)* | 24 (33.3%) |
| bb | 50 (50%) | 6 (7.7%)‡ | 21 (29.2%)† |
| ApaI | | | |
| AA | 50 (50%) | 21(26.9%)‡ | 18 (25%)‡ |
| Aa | 20 (20%) | 45 (57.7%)‡ | 45 (62.5%)‡ |
| aa | 30 (30%) | 12 (15.4%)‡ | 9 (12.5%)‡ |
| FokI | | | |
| FF | 60 (60%) | 18 (23.1%)‡ | 27 (37.5%)† |
| Ff | 20 (20%) | 33 (42.3%)† | 42 (58.3%)‡ |
| ff | 20 (20%) | 27 (34.6%)* | 3 (4.2%)‡ |
| TaqI | | | |
| TT | 60 (60%) | 36 (46.2%)‡ | 30 (41.7%)‡ |
| Tt | 30 (30%) | 21 (26.9%) | 27 (37.5%) |
| tt | 10 (10%) | 21 (26.9%)† | 15 (20.8%)* |

Data are shown as number and percentage. Significance levels: *P≤0.05, †P≤0.01, ‡P≤0.001 vs control group (chi-square test for pair-wise proportions).

Table 4. Correlation between 25(OH)D level with various biochemical and clinical findings

| | 25(OH)D |
|--------------------------|--------------------|
| Waist circumference | $r = -0.5\ddagger$ |
| Total calcium | $r = 0.6\ddagger$ |
| Total cholesterol | $r = -0.5\ddagger$ |
| Triglycerides | $r = -0.6\ddagger$ |
| Systolic blood pressure | $r = -0.1$ |
| Diastolic blood pressure | $r = -0.5\ddagger$ |
| Fasting blood glucose | $r = -0.5\ddagger$ |
| Postprandial glucose | $r = -0.6\ddagger$ |

Significance levels: $\ddagger P \leq 0.001$ of Pearson's correlation coefficient.

($P \leq 0.001$), postprandial blood glucose level ($P \leq 0.001$), triglycerides ($P \leq 0.001$), diastolic blood pressure ($P \leq 0.05$), and waist circumference ($P \leq 0.001$). Other genotypes of VDR had no significant association with any of the clinical or laboratory findings in the patients (data not shown).

DISCUSSION

There is evidence that VDR genotype may affect insulin action by regulating its secretion and resistance to its action [23].

Table 5. Association of genetic FokI polymorphism with various clinical and biochemical findings in both diabetic patients and patients with metabolic syndrome collectively

| | FokI polymorphism | | |
|---------------------------------|-------------------|----------------|---------------|
| | FF (n=45) | Ff (n=75) | ff (n=30) |
| Waist circumference (cm) | 98.90 ± 10.5† | 105.00 ± 12.4† | 98.7 ± 2.4† |
| Systolic blood pressure (mmHg) | 111.38 ± 6.6 | 112.8 ± 7.2 | 110.7 ± 8.0 |
| Diastolic blood pressure (mmHg) | 78.6 ± 6.4* | 98.7 ± 13.0* | 84.1 ± 6.2* |
| Total calcium (mg/dl) | 8.8 ± 0.67* | 8.2 ± 0.71* | 8.5 ± 0.74* |
| Ionized calcium (mg/dl) | 4.08 ± 15 | 4.01 ± .15 | 4.04 ± 17 |
| 25(OH)D (nmol/l) | 91.5 ± 31.4* | 83.0 ± 26.6* | 73.02 ± 19.2* |
| Total cholesterol (mg/dl) | 208.38 ± 45.7‡ | 268.7 ± 45.5‡ | 225.7 ± 38.7‡ |
| HDL-cholesterol (mg/dl) | 49.9 ± 5.37† | 47.4 ± 7.5† | 33.0 ± 6.1† |
| Triglycerides (mg/dl) | 169.1 ± 41.4‡ | 205.4 ± 58.2‡ | 200.0 ± 52.7‡ |
| Fasting blood glucose (mg/dl) | 169.9 ± 60.6 | 192.7 ± 39.9 | 188.4 ± 54.4 |
| Post prandial glucose (mg/dl) | 197.8 ± 73.49‡ | 235.7 ± 69.2‡ | 225.5 ± 56.3‡ |

Data are shown as mean ± SD. Significance levels: * $P \leq 0.05$, † $P \leq 0.01$, ‡ $P \leq 0.001$ (ANOVA).

Study of VDR genetic polymorphism had shown significant increase in the prevalence of Ff genotypes among diabetic patients and patients with signs of metabolic syndrome. Similar results were reported previously supporting those genotypes alleles in FokI gene of VDR can be associated more frequently in diabetic patients [24-27]. On contrary to our results, previous results on Egyptian patients with type 2 DM and metabolic syndrome revealed more frequent association of FF genotypes among those patients [28]. Also, previous reports had contradictory results about the association of polymorphism of VDR genes FokI, ApaI, BsmI and TaqI and risk of type 2 DM [29]. The reason for the discrepancy among the results may reflect the genetic differences in the studied populations and the interaction with other environmental factors predisposing to type 2 DM. Although mechanistically unclear, it has been suggested that both environmental and genetic factors seem to be involved in type 2 DM development [4].

There were significant associations between Ff VDR genotype and BMI ($P \leq 0.01$), and waist circumference ($P \leq 0.001$). Polymorphisms in the VDR gene have been linked with the increased susceptibility to obesity in subjects with type 2 DM [30-32]. Moreover, the results of the present study demonstrates significant association between Ff genotype of VDR and both elevated lipid parameters and diastolic blood

pressure. As known, the gene for VDR is also expressed on different tissues and mediates the action of vitamin D. Previous studies have reported the association of FokI VDR polymorphism with altered lipid profile [15]. It has been also reported that lower serum 25(OH)D may be associated with higher lipid parameters in diabetic patients. The present study provides a supportive evidence for this relation as there was significant negative correlation between 25(OH)D and both total cholesterol and triglyceride levels in both diabetic patients and patients with metabolic syndrome.

An explanation for such finding may include the increased secretion of parathyroid hormone secondary to declining levels of vitamin D. The reduced vitamin D level is associated with decrease in intestinal calcium absorption with the resultant increase in parathyroid hormone secretion and subsequent acceleration of lipolysis [33]. Likewise, the reduced vitamin D level together with decreased intestinal calcium absorption can lead to increase in the hepatic triglyceride formation and secretion [34]. Moreover, the reduced vitamin D level decreases insulin secretion and insulin sensitivity that affects lipid metabolism [35].

One remarkable finding of the present study was to find a negative correlation between BMI and waist circumference and vitamin D level. In line with this observation, some previous studies have also reported correlation between decreased vitamin D levels and increase in BMI and waist circumference [36-38]. Also between vitamin D deficiency and increase of body fat [39, 40].

Vitamin D deficiency has been reported as a risk factor for various diseases such as osteoporosis, autoimmune diseases, various types of cancer, and cardiovascular diseases [41-44]. This association can be attributed to the common etiology of both obesity and reduced vitamin D due to reduced outdoor exercise. However, the results concerning this association had shown variations [45, 46]. Thus the nature of this association has to be fully investigated to understand this intimate orchestration as central adiposity is a key driver in the development of metabolic syndrome as well as cardiovascular disorders [47]. The association of reduced vitamin D level with increased diastolic blood pressure may be attributed to the vitamin D effects on lipid profile [48, 49].

There was significant negative correlation between 25(OH)D and both fasting and post-prandial blood glucose levels. There is evidence that supports an effect of vitamin

D on multiple levels of insulin release and action. Insulin release has been shown to be low in vitamin D-deficient rats and is enhanced by treatment with 1,25(OH)D [50, 51]. This may be attributed to the complex effects of vitamin D on protein synthesis and/or increased conversion of pro-insulin to insulin [52].

In conclusion, the data obtained in this study show that the genetic polymorphism of vitamin D receptor might play a role in the development of type 2 DM and metabolic syndrome. Moreover, the serum level of 25(OH)D appears to have negative association with lipogram parameters such as total cholesterol and triglycerides. Despite the limitations of this study, we think this little piece of research might provide useful information on the association between VDR polymorphism and the risk of type 2 DM and/or metabolic syndrome in a sample of Egyptian patients. Larger multicenter studies with longer duration would be indeed more useful to consider limitations of data in hand and their context in comparison with those of other similar studies.

Conflict of interest

The authors declare that they have no conflict of interest.

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