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Original article

Role of vitamin D and vitamin D receptor gene polymorphisms on residual beta cell function in children with type 1 diabetes mellitus

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ABSTRACT

Background: After the onset of type 1 diabetes mellitus (T1DM), preservation of the residual ß-cell function can help good metabolic control. The aim of this study was to evaluate the effect of vitamin D and its receptor gene polymorphisms on residual ß-cells function.
Methods: One hundred and one children with T1DM (new cases) older than 5 years were selected. Vitamin D receptor (VDR) gene polymorphisms, vitamin D (VD), fasting and stimulated C-peptide (FCP and SCP) levels were measured within 1.5 and 4.5 month after the diagnosis of disease. Kruskal-Wallis and Mann-whitney U test were used for comparing the study groups. Generalized estimating equation (GEE) model was used for the estimation of association between VD and VDR gene polymorphisms with FCP and SCP after adjustment for comorbid variables.
Results: The most frequent genotypes and alleles in TaqI, FokI, BsmI and ApaI polymorphisms were TT (50%) and allele T (68.88%), FF (59.2%) and allele F (77.04%), Bb (41.8%) and allele b (61.73%), and Aa (53.1%) and allele A (63.29%) respectively. In children with higher VD levels, the C-peptide (CP) levels were elevated. Also we observed: the tt genotype associated with increasing SCP levels compared with TT genotype; the bb and Bb genotypes were associated with increasing both FCP and SCP in comparison to BB; and the aa and Aa genotypes were associated with decreasing FCP in comparison to the AA genotype.
Conclusions: Sufficient levels of VD (more than 30 ng/ml) can preserve residual ß-cells and insulin secretion.

Introduction

Type 1 Diabetes Mellitus (T1DM) is a result of an autoimmune process. Various factors collectively result in destruction of pancreatic ß-cells by immune system, consequently causing decrease in insulin secretion and hyperglycemia. In the event of poor metabolic control, end organ damage (retinopathy, nephropathy, neuropathy and macrovascular complications) will ensue over time. T1DM affects approximately 90% of children and adolescents with diabetes [1]. The prevalence of T1DM is increasing in most countries [1]. Since diabetes is a life time disease, preventing and delaying its complications will help to improve the patients' quality of life. At the time of clinical presentation, there is still small amount of ß-cell mass that secretes insulin. However, the mass of the remaining ß-cells is lower in children, since the earlier diabetes manifests in life, the genetic predisposing factors are stronger and the damage will be more severe [2,3]. Endogenous insulin helps better control of blood glucose levels and can be effective in preventing complications of diabetes [4]. Many studies have been conducted to preserve the function of residual ß-cells following the onset of diabetes; T1DM is a result of numerous genetic and environmental factors, and identifying them can help its prevention. Vitamin D deficiency is

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known as one of the environmental factors that make individuals prone to various autoimmune diseases including T1DM [3,5]. The anti-inflammatory properties of vitamin D (VD) are known. Vitamin D binds to its intracellular receptor (VDR), and induces transcription of its target genes. Approximately 2700 VDR-binding sites exist in the genome which explains the numerous effects of VD. Apart from its effects on mineral metabolism, VDR has anti-inflammatory properties and modulates the immune system. VDR is also present in pancreatic ß-cells and stimulates insulin secretion [6]. The VDR gene is a pleiotropic gene which contains several single nucleotide polymorphisms (SNPs), some of which affect receptor function. Four of these SNPs include TaqI, BsmI, FokI and ApaI, which have been investigated for their association with increasing the risk of diabetes. FokI is located in exon 2, and the F allele results in the production of a protein with 424 amino acids, which is more active against the longer protein (427 amino acids) produced by the f allele. ApaI and BsmI variants are located in intron 8, and TaqI is located in exon 9. These 3 polymorphisms are near the end of the 3′UTR (untranslated region) of the gene, which does not alter the structure of the resulting protein, but rather changes the stability of mRNA [6,7].

There are some inconsistencies in relationship between specific polymorphism of VDR gene and incidence of diabetes in different studies that maybe related to the ethnic differences [7–16]. In a meta-analysis, Tizaoui et al reported that several haplotypes of the VDR gene increase the risk of diabetes more than a single polymorphism alone. Additionally, haplotypes interact with environmental factors and affect susceptibility to diabetes [17].

The role of VD deficiency in the onset of diabetes has been confirmed. Several studies have also been conducted on the effect of VD on preserving residual ß-cells in newly diagnosed patients with diabetes, but their results have not been conclusive due to inconsistency [3–5,18–20]. VDR gene polymorphism can affect VDR function, which probably explains diversity of the results from clinical studies. The aim of this study was to assess the relationship between VD level and polymorphisms of the VDR gene in residual ß-cell preservation function in order to understand the role of VD (as an environmental factor) and VDR gene polymorphism (a genetic factor) in endogenous insulin production and improvement in diabetes management.

Materials and methods

Study design and population

This was a prospective cohort study including 101 patients with T1DM (persian ethnic), diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) criteria, ages 5 years and older, who were affected by diabetes for less than 1.5 months [21]. The subjects were selected among patients who referred to the Diabetes Clinic or newly admitted cases to the Endocrinology Ward at the Children's Medical Center (CMC) from August 2016 to April 2017. The study was approved by the Ethics Committee of the Faculty of Medicine prior to implementation.

Clinical evaluations

The children's heights were taken in a standing position, without shoes, using German-manufactured Secastadiometer in the standard position and weights were taken without heavy clothing by seca scale. The Body Mass Index (BMI) was calculated using the wt (kg) / ht^2 (m^2) equation, and the BMI curve was adjusted for age and sex according to National Center for Health Statistics (NCHS) growth charts. Patients were divided into 4 groups: underweight (< 5 percentile), normal (5–85 percentiles), overweight (85–95 percentile), and obese (> 95 percentile). The presence of diabetic ketoacidosis (DKA) at the time of diagnosis and other autoimmune diseases were evaluated based on laboratory test results and hospital records. The severity of DKA was determined as either mild (venous PH: 7.2–7.3; HCO3:10-15 mmol/l), moderate (venous PH: 7.1–7.2; HCO3:5-10 mmol/l) or severe (venous PH < 7.1; HCO3 < 5 mmol/l), according to the ISPAD 2014 guidelines [22].

Laboratory evaluations

Following sample selection and completion of questionnaires with clinical and demographic information, VD level, Fasting C-Peptide (FCP) and Stimulated C-Peptide (SCP) levels were measured 1.5 months after diagnosis of diabetes. If the FCP or SCP level was greater than 0.6 ng/ml, VD, FCP and SCP levels were re-measured in 4.5 months following the diagnosis of diabetes.

Biochemical analysis

The VD levels were measured using Chemiluminescent methods with the ARCHITECT kit manufactured in Ireland. FCP levels were measured following 8 h of fasting and at least 6 h from the last injection of insulin; while SCP levels were taken 2 h post breakfast and without insulin injection using Immunoassay methods with a Monobind Kit Manufactured in China under a USA License.

Genomic DNA extraction and genotyping

Genomic DNA was isolated from samples collected in EDTA tubes using phenol chloroform protocol. Genotyping for VDR gene polymorphisms in the patients were determined using polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) analysis as summarized in (Table 1).

Table 1
Primers sequence, annealing temprerature and fragments results from enzymatic digestion of PCR products.

Variant type	Primer sequence		Annealing Temperature	Fragments
FokI	Forward Reverse	5′- CACTGACTCTGGCTCTGACCGT-3′ 5′-AACACCTTGCTTCTTCTCCCTCC-3′	68.1 °C	T/T: 192, 58 bps T/C: 250, 192, 58 bps C/C: 250 bps
TaqI*	Forward Reverse	5′-CAGAGCATGGACAGGGAGCAA -3′ 5′- GCAACTCCTCATGGCTGAGGTCTC -3′	68 °C	T/T: 494, 251 bps T/C: 494, 251, 293,201 bps C/C: 251, 293,201 bps
ApaI*	Forward Reverse	5′-CAGAGCATGGACAGGGAGCAA -3′ 5′- GCAACTCCTCATGGCTGAGGTCTC -3′	68 °C	G/G: 217, 528 bps G/C: 217, 528, 745 bps C/C: 745 bps
BsmI	Forward Reverse	5′-AAGGAGACACAGATAAGGAAATAC -3′ 5′-TCCAAAATCAATCAGGTAAACTA -3′	61 °C	A/A: 317bps G/A: 317, 140, 177bps G/G: 177,140 bps

Statistical analysis

The assumption for the normal distribution of the study variables was made using the Kolmogorov-Smirnov test. Quantitative variables were defined using mean +/- standard deviation (SD) or median (25th– 75th percentiles), and qualitative variables were described with numbers (percentages). In order to analyze the comparison of CP levels among different groups according to VD status (sufficient: > 30 ng/ml, insufficient: 10–30 ng/ml, and deficient: < 10 ng/ml) [23], the Kruskal-Wallis test was used. A comparison between any two groups was performed using the Mann-Whitney test (with Bonferroni correction of *p*-value). To investigate the relationship between changes in VD levels (at the beginning and end of the study) with changes in CP levels after controlling for all other variables (age, duration between the first and second measurements of CP, the patient's condition with regards to ketoacidosis at the time of diagnosis, and the various genotypes of BsmI, TaqI, FokI and ApaI polymorphisms) the General Estimating Equation (GEE) model was used. A *p*-value of less than 0.05 ($\alpha < 0.05$) was considered statistically significant, and all statistical analyses were performed using the STATA software, version 13.

Results

One hundred and one patients with T1DM were evaluated. The range (min and max), mean (SD) age of the patients were 10 (5 and 15), 9.28 (2.54) years respectively. Fifty four patients had DKA presentation at onset of disease (Table 2). Thirty s even patients (38.5%) had VD deficiency (25(OH) D3, <10 ng/ml; F/M:23/14) at the start of the study, 47 (49%) patients had insufficient (25(OH) D3 ≥ 10, <30 ng/ml; F/M:17/30), and only 12 (12.5%) patients had 25(OH) D3 levels within the normal range of 30–60 ng/ml(F/M:7/5).The association between VD level and gender state was statistically significant ($p = 0.04$). Minimum, maximum, and mean (SD) of FCP were 0.1, 2, 0.69 (0.42) ng/ml respectively. Two patients had FCP higher than 1.9 ng/ml (maximum of laboratory reference intervals), BMI in these patients were lower than 85 percentile.

Alleles and genotypes of VDR polymorphisms

In this study, the alleles and genotypes frequencies of VDR gene at positions TaqI, FokI, BsmI and ApaI were examined (Table 3). As shown in Table 3, the most frequent genotypes and alleles in TaqI, FokI, BsmI and ApaI polymorphisms were TT (50%) and T (68.88%), FF (59.2%) and F (77.04%), Bb (41.8%) and b (61.73%), and Aa (53.1%) and A (63.29%) respectively. Analysis of deviations from Hardy-Weinberg equilibrium demonstrated that all the polymorphism genotypes were in the equilibrium ($p > 0.05$). As shown in Table 4, there was no association between genotypes and alleles frequencies of VDR gene polymorphisms and baseline vitamin D level ($p > 0.05$). After adjusting for age, sex, and BMI, these associations were not statistically significant in binary logistic regression models ($p > 0.05$).

Table 2
Baseline characteristics of the study population (n = 101).

Variables		
Age, year, mean (SD)		9.28 (2.54)
Gender, Female/male		51/50
BMI, percentile, n (%)	<5	24 (23.8)
	5-85	71 (70.3)
	85-95	6 (5.9)
	>95	0 (0)
DKA presentation, n (%)	Mild	25 (24.75
	Moderate	15 (14.85)
	Sever	14 (13.87)
Non- DKA presentation, n (%)	47 (46.53)	
Autoimmune disease comorbidity, n (%)	All	6 (5.94)
	Hypothyroidism	3 (2.97)
	Celiac disease	2 (1.98)
	Alopecia areata	1 (0.99)
Non- Autoimmune disease comorbidity, n (%)	89 (88.12)	

Table 3
Distribution, genotypes and alleles frequencies of VDR gene polymorphisms in patients with T1DM.

Patient		Polymorphism	
Percent	Frequency		
59.2%	58	**FF**	**FokI**
35.7%	35	**Ff**	**Genotype**
5.1%	5	**ff**	
77.04%	151	**F**	**FokI**
22.96%	45	**f**	**Allele**
17.3%	17	**BB**	**BsmI**
41.8%	41	**Bb**	**Genotype**
40.8%	40	**bb**	
38.37%	75	**B**	**BsmI**
61.73%	121	**b**	**Allele**
50%	49	**TT**	**TaqI Genotype**
37.8%	37	**Tt**	
12.2%	12	**tt**	
68.88%	135	**T**	**TaqI**
31.12%	61	**t**	**Allele**
37.8%	37	**AA**	**ApaI**
53.1%	52	**Aa**	**Genotype**
9.2%	9	**aa**	
63.29%	126	**A**	**ApaI**
35.71%	70	**a**	**Allele**

The residual β-cell function

Vitamin D, FCP and SCP were evaluated in patients at 1.5 and 4.5 month after the diagnosis (Fig. 1 and Table 5). As shown in Fig. 1 and Table 5, in overall and at each evaluation time, the FCP and SCP levels in patients with sufficient VD level were higher than insufficient and deficient levels of VD and these differences were statistically significant for SCP ($p = 0.02$).

After adjusting for age, BMI, time, and DKA state, the association between VD level and VDR polymorphisms genotypes with SCP and FCP were evaluated separately in GEE models. Type 1 diabetes patients with sufficient VD levels had higher SCP than patients with deficient levels [0.79 ng/ml (CI 95%: 0.38–1.21; $p < 0.001$)]. Three genotypes of VDR polymorphisms were associated with higher levels of SCP (tt *vs*. TT and bb and Bb *vs*. BB) ($p < 0.05$) (Table 6). Although the ff *vs*. FF genotype was associated with lower level of SCP, but this difference was not statistically significant ($p = 0.21$). There was no association between VD level and FCP ($p = 0.32$). Two genotypes of VDR polymorphisms were associated with higher levels of FCP (bb and Bb *vs*. BB) and two genotypes of VDR polymorphisms were associated with lower levels of FCP (aa and Aa *vs*. AA) ($p < 0.05$) (Table 6).

Patients with DKA presentation at the onset of disease showed lower level of SCP than patients without DKA: in patients with mild DKA (0.44 ng/ml; CI 95%: 0.05-0.83; $p = 0.003$), moderate DKA (0.85 ng/ml; CI 95%: 0.3-0.4; $p = 0.03$) and severe DKA (0.9 ng/ml; CI 95%: 0.41–1.4; $p < 0.001$). Patients with severe DKA at the onset of disease, had lower levels of FCP than patients without DKA (0.35 ng/ml; CI 95%: 0.13-0.57; $p = 0.002$).

Table 4
Genotypes and alleles frequencies of VDR gene polymorphisms stratified by baseline vitamin D level.

Polymorphism	Genotype	Vitamin D level			Total (n = 94)	*p* value[†]
		Defficient (n = 36)	Insufficient (n = 46)	Sufficient (n = 12)		
FokI	**FF**	21 (38.2%)	26 (47.3%)	8 (14.5%)	55 (100%)	**0.17**
	Ff	15 (44.1%)	17 (50%)	2 (5.9%)	34 (100%)	
	ff	0 (0%)	3 (60%)	2 (40%)	5 (100%)	
BsmI	**BB**	6 (35.3%)	10 (58.8%)	1 (5.9%)	17 (100%)	**0.93**
	Bb	15 (37.5%)	17 (42.5%)	8 (20%)	40 (100%)	
	bb	15 (40.5%)	19 (51.4%)	3 (8.1%)	37 (100%)	
TaqI	**TT**	21 (45.7%)	20 (43.5%)	5 (10.9%)	46 (100%)	**0.33**
	Tt	11 (29.7%)	20 (54.1%)	6 (16.2%)	37 (100%)	
	tt	4 (36.4%)	6 (54.5%)	1 (9.1%)	11 (100%)	
ApaI	**AA**	15 (41.7%)	17 (47.2%)	4 (11.1%)	36 (100%)	**0.6**
	Aa	17 (34%)	25 (50%)	8 (16%)	50 (100%)	
	aa	4 (50%)	4 (50%)	0 (0%)	8 (100%)	

[†] chi-square test, after combining of sufficient and insufficient vitamin D levels subgroups.

There was no statistically significant association between DKA presentation at onset of T1DM disease and each of four VDR polymorphisms genotypes ($p > 0.05$). Diabetic ketoacidosis (DKA) incidence at the onset of disease in patients with and without VD deficiency were 45.9% and 19.3% respectively ($p = 0.006$).

Discussion

The residual ß-cell function in T1DM is considered based on CP levels which might be detectable in blood for a few decades after diagnosis. Any factor which increases the insulin production potential is capable of improving metabolic control of the disease and preventing its complications [24]. These include both environmental and genetic factors. Our study is among the first studies which has evaluated the effect of both factors. Based on the current study, it has been demonstrated that VD is one of the factors which is important in insulin production. We did not find any significant associations between the genotypes of VDR polymorphisms and VD deficiency. This finding is inconsistent in studies and suggest that these SNPs may influence vitamin D levels rather differently in various populations [25].

VD and CP levels were measured twice during this study (1.5 and 4.5 months from the time of T1DM diagnosis), and the findings showed that with an increase in VD level, CP level was increased as well. Nearly all immune cells express the VDR.VD has both pro- and anti-inflammatory actions [26]. VD decreases inflammatory cytokines production including IL-6, reduces inflammation and infiltration of T-cells and as a result, controls the autoimmune process. At the level of immune cells, VD inhibits the differentiation and maturation of dendritic cells and stimulates their apoptosis, preventing their transformation to antigen presenting cells, which is the first step in the immune response. Vitamin D protects T suppressor cells, which in turn, decreases the Th1 cytokine production that has a role in destruction of ß-cells. Moreover, VD shifts the immune response toward Th2 pathway, creating benign insulitis [1,7,26]. In fact, VD has an anti-apoptotic effect on pancreatic ß-cells by controlling apoptosis-inducing cytokines. Vitamin D also induces and preserves high levels of A20 gene protein, which decreases nitric oxide (NO) levels. NO directly induces dysfunction and loss of ß-cells, while indirectly provokes the expression of the Fas receptor (a transmembrane cell surface receptor and a member of the tumor necrosis factor (TNF) receptor family). The apoptosis resulting from the Fas ligand is seen in pancreatic ß-cells of patients with T1DM [1,7]. Other studies have also reported similar results in agreement with our finding in this study [3,5,18,27,28]. However, in previous studies genetic factors were not investigated, while our study revealed the relationship between VD and CP considering the role of VDR polymorphisms. On the other hand, none of the previous studies had evaluated the correlation between VD and CP levels [19,20,29].

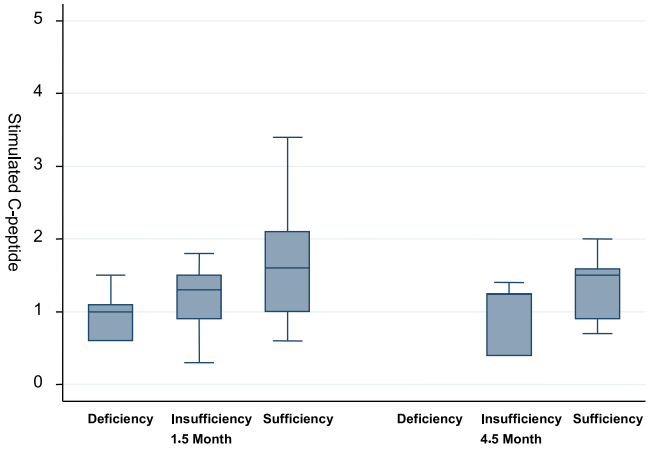

Fig. 1. Stimulated C-peptide levels in patients with Type 1 diabetes mellitus according to the vitamin D levels.

In our study which has been conducted on 101 children with T1DM referred to the CMC in Tehran – Iran, it has been determined that in children with higher VD levels and without DKA at the time of diagnosis, the CP levels were also elevated. Furthermore, the genetic evaluation of the VDR gene showed a statistically significant relationship between certain VDR gene polymorphisms and their CP levels.

Unfortunately, in our study, 87.5% of patients had a VD level below 30 ng/ml (VD insufficiency and deficiency). Many observational studies have reported the high incidence of VD deficiency in patients with diabetes [1,30]. In a 2016 study in Egypt, 70% of Egyptian children with T1DM, and a mean disease duration of 4.11 (SD of ± 2.3) years had VD deficiency [31]. Various studies in Iran have also shown a high prevalence of VD deficiency. In one study in Isfahan, 26% of children between age 6–7 years-old had a VD level below 33 ng/ml [32]. Razzaghi Azar et al. reported a VD level of less than 20 ng/ml in 78% of healthy children age 8–18 years-old in Tehran [33]. In another study in Tehran, 91.7% of healthy children age between 9–12 years had a VD level of less than 20 ng/ml in the fall and winter [34]. Ataie et al. who had examined

Table 5
Stimulated and fasting C-peptide levels at the 1.5 and 4.5 month after type 1 diabetes mellitus diagnosis stratified by vitamin D level.

C-peptide	Vitamin D level	Total	Time after diagnosis	
			1.5 month	4.5 month
Stimulated C-peptide, ng/ml, Median (Q25-Q75)	**Deficient**	1 (0.6-1.25)	0.6 (1-1.25)	–––––
	Insufficient	1.28 (0.75-1.5)	0.85 (1.3-1.55)	0.4 (1.24-1.33)
	Sufficient	1.5 (0.95-1.9)	0.99 (1.6-2.15)	0.9 (1.5-1.6)
***p* value**†		**0.02**	**0.01**	**0.01**
Fasting	**Deficient**	0.6 (0.3-0.8)	0.55 (0.38-0.83)	–––––
C-peptide, ng/ml, Median (Q25-Q75)	**Insufficient**	0.7 (0.3-1)	0.7 (0.3-1.03)	1.24 (0.4-1.33)
	Sufficient	0.75 (0.48-0.95)	0.75 (0.48-0.95)	1.5 (0.9-1.6)
***p* value**†		0.45	0.71	0.21

† Kruskal Wallis test.

Table 6
Association between vitamin D receptor polymorphisms and fasting and stimulated C-peptide in general estimating model.

Polymorphism	Genotype	Stimulated C-peptide		Fasting C-peptide	
		Coefficeint (CI 95%)	*p* value	Coefficeint (CI 95%)	*p* value
FokI	**FF**	Referent group		Referent group	
	ff	−0.39 (-0.98,0.21)	0.2	−0.41 (-0.89,0.06)	0.09
	Ff	0.3 (-0.11,0.7)	0.15	0.17 (-0.02,0.36)	0.08
BsmI	**BB**	Referent group		Referent group	
	bb	1.63 (0.68,2.59)	**0.001**	0.59 (0.09,1.09)	**0.02**
	Bb	1.16 (0.61,1.71)	<**0.001**	0.4 (0.1,0.7)	**0.009**
TaqI	**TT**	Referent group		Referent group	
	tt	1.58 (0.56,2.6)	**0.002**	0.55 (-0.04,1.14)	0.07
	Tt	0.23 (-0.22,0.68)	0.31	0.18 (-0.11,0.47)	0.23
ApaI	**AA**	Referent group		Referent group	
	aa	−0.009 (-0.51,0.49)	0.98	−0.33 (-0.02,-0.64)	**0.03**
	Aa	−0.008 (-0.55,0.53)	0.98	−0.31 (-0.08,-0.54)	0.007

VD levels in newly diagnosed children with diabetes between the age 8–18 years during the fall and winter month in Tehran, observed that 77% of patients had VD levels below 20 ng/ml, and 23% had VD levels of 20–30 ng/ml. In other words, 100% of the patients in their study had VD levels below 30 ng/ml. Given the role of VD deficiency as a potential environmental factor in autoimmune disorders, including T1DM, the evaluation of VD levels and treatment of deficiency in susceptible individuals is essential. Various studies have demonstrated that ordinary diet alone cannot supply the necessary amount of VD, therefore, enriching foods with VD, especially in winter, can help to provide the physiological amount of VD required for the body [35].

In addition to environmental factors, genetic factors also play important role in survival of ß-cells and for maintaining insulin production. Among the genetic factors involved in this process VDR gene polymorphisms are interesting markers. In this study, the researchers noted that F allele and FF genotype, b allele and Bb genotype, T allele and TT genotype, and A allele and Aa genotype were more frequent and the following genotypes affected FCP and SCP as noted: the tt genotype increased SCP levels when compared to the TT genotype; the bb and Bb genotypes increased both FCP and SCP in comparison to BB; and the aa and Aa genotypes decreased FCP in comparison to the AA genotype. In all of these analyses, the effect of other variables, such as age and BMI, were adjusted. In other word, the tt bb genotypes were associated with higher SCP levels, while the AA, bb and Bb genotypes were linked with higher FCP. Animal studies have previously shown the VDR gene destruction effect on ß-cell function [36]. In a study by Bogdanou et al., following treatment with VD, the T-regulatory cells were increased in the carriers of some specific genotypes of the VDR gene polymorphism. However, they did not reach a conclusion as further studies were needed to examine the relationship between ß-cell function and VDR genotypes [24].One study indicates that higher concentration of VD in combination with minor alleles at VDR rs7975232 (aa genotype), may decrease risk of islet autoantibody in children who were at increased risk for T1DM [26]. Therefore, it is likely that polymorphism of the VDR gene also plays a role in regulating the immune system, and might be effective in slowing down the autoimmune process and destruction of ß-cells. The only human study that was carried out on the relationship between VDR gene polymorphism and ß-cell function was reported by Moryet al. They observed that patients with T1DM carrying f allele of FokI polymorphism had lower FCP levels, while the different genotypes of the BsmI polymorphism showed no association with ß-cell function. In their study, they showed that the ratio of individuals with FCP > 0.6 ng/ml was 5.8% in subjects with the ff and Ff genotypes, and 14.3% in those with the FF genotype. However, the observed difference was not statistically significant ($p = 0.07$) [37]. Similarly, in our study a relationship between CP levels and FokI polymorphism was not detected but it was found that CP levels were changed among various genotypes of the BsmI polymorphism. Preserving the residual function of ß-cells has a polygenic basis and it is clearly evident that the relationship between polymorphisms of the VDR gene and CP levels is a complex issue, and causality and consistency in this relationship should be confirmed by further studies.

In the context of the association between the VDR gene polymorphism and complications of diabetes, Bonakdaran et al. reported the association of the Ff genotype with DKA [11]. Another study showed that the F allele was associated with a lower prevalence of diabetic retinopathy [38]. Since the patients in our study were all newly diagnosed cases of diabetes, the relationship

between VDR polymorphism and the incidence of diabetes complications could not be verified.

Our study is among the first studies to evaluate VD status and VDR gene polymorphisms on pancreatic ß-cell function in children with T1DM.

This study also had certain limitations: First, the number of subjects evaluated for the different genotypes of polymorphisms in the VDR gene were few, therefore, rendering accurate examination of all genotypes as well as investigation of the interaction between VD and various polymorphisms of the VDR gene was not conclusive. Second, it was not an interventional study. Third, the follow-up period was relatively short and the long-term evaluation of these patients was not possible. Additionally, the researchers could not assess, account for, or eliminate the effect of autoimmune factors. Nevertheless, the investigators made every effort to adjust for other variables, such as age, sex, and BMI through accurate follow-up of patients and appropriate use of statistical methods. Despite these efforts, the role of residual confounding factors cannot be entirely ignored.

Conclusion

This study showed that VDR gene polymorphism and VD play a role in the control and progression of T1DM, and higher VD levels have a protective effect on pancreatic ß-cells. Given that diabetes is a multifactorial disease, it is clear that a single treatment alone is not effective enough for secondary prevention. Therefore combination of several treatments may be required. Vitamin D supplementation coupled with other factors (that should be identified in the future studies), might be capable of secondary prevention of diabetes, and improve the quality of life of patients by reducing the complications of the disease.

Authors' contribution

N. Habibian: Literature search and data collection; M.M. Amoli: Study design and acceptance of final manuscript version; F. Abbasi: Study design and acceptance of final manuscript version; A. Rabbani: Data interpretation and fund collection; A. Alipour: Statistical analysis; F. Sayarifard: Data interpretation; P. Rostami: Data interpretayion; S.P. Dizaji: Data interpretation; B. Saadati: Statistical analysis; A. Setoodeh: Study design and acceptance of final manuscript version.

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