Relationship between vitamin D receptor gene polymorphisms and vitamin D levels in children

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Ethics Committee Approval
Approval for this study was obtained from the Ethics Committee of Kafkas University Faculty of Medicine with the decision number 11 of the document dated 31.10.2018 and numbered 177.

Abstract

Background/Aim: Vitamin D exerts its effects in the body through its receptors. Polymorphisms in vitamin D Receptor (VDR) gene are known to cause certain diseases and affect vitamin D levels. In this study, we planned to examine the relationship between vitamin D levels and Vitamin D gene polymorphisms among children.

Methods: The study group included 124 healthy children living in the same region. Vitamin D (VitD), Parathyroid Hormone (PTH), Alkaline phosphatase (ALP), Calcium (Ca), Phosphorus (P) and Magnesium (Mg) were examined in the blood samples taken. In terms of measured Vitamin D levels, children were divided into group 1 (Vitamin D < 20 ng/mL) and group 2 (Vitamin D ≥ 20 ng/mL). Deoxyribonucleic Acid (DNA) was isolated from the serum sample, VDR ApaI and TaqI polymorphisms were determined by Polymerase Chain Reaction (PCR) method, and comparisons were made between groups.

Results: The overall mean age of the children included in the study was 8.11 (4.98) years. The mean ages of participants in Groups 1 and 2 were 9.38 (4.87) years and 6.38 (4.62) years, respectively (P=0.091). The mean vitamin D levels of Groups 1, 2 and overall were 13.82 (3.29) ng/mL, 33.96 (20.47) ng/mL, and 23.49 (21.54) ng/mL, respectively (P=0.509). The two groups were similar in terms of serum ALP, PTH, Ca, Mg and P levels (P>0.05 for all). VDR polymorphisms were found to have no effect on Vitamin D levels.

Conclusion: In our study, no relationship was found between the genotypes of ApaI and TaqI polymorphisms and Vitamin D levels. This study is important in terms of remarking the fact that Vitamin D deficiency is still a public health problem and its contributions to VDR gene polymorphism research.

Keywords: Vitamin D, Vitamin D receptor, Polymorphism
Introduction

Vitamin D is mainly synthesized in the skin by way of ultraviolet-B (UV-B) rays from sunlight, but also partially ingested from vegetable and animal foods. It is activated in our body after being hydroxylated in the liver first, then the kidney, and besides its many effects in our body, it mainly affects the calcium (Ca⁺²) metabolism [1,2]. It was demonstrated that Vitamin D receptor (VDR) resides in the immune system, as well as many tissues such as blood cells and the central nervous system [3].

The function of Vitamin D in our body is mediated by VDR. Many polymorphisms were identified in studies examining the VDR gene, i.e., ApaI, TaqI, FokI and BsmI polymorphisms [4]. There are studies investigating the relationship between Vitamin D levels and VDR gene polymorphisms, and different results have been obtained in different populations [5-9].

In our study, we aimed to determine the frequency of VDR Apa and Taq polymorphisms, their genotype typology in healthy children and relationship with serum vitamin D levels.

Materials and methods

A total of 124 healthy children aged 12 months-18 years who visited Kafkas University Faculty of Medicine, General Pediatrics Outpatient Clinic for routine checkup, had no complaints and normal physical examination findings, had not used daily or depot vitamin D preparations within the last 3 months and not lived in any place other than the place of residence within the last 6 months were included in this study. A total of 72 children with vitamin D insufficiency, deficiency and severe deficiency based on blood samples obtained in routine examinations, and 52 children with normal vitamin D values were included in the study on a voluntary basis.

Approval for this thesis study was obtained from the Ethics Committee of Kafkas University Faculty of Medicine with the decision number 11 of the document dated 31.10.2018 and numbered 177. Informed consent was obtained from each participating child and their parents by providing detailed information about the study.

Those with a condition requiring hospitalization, without an informed consent form obtained from their parents and those who did not want to participate in the study, those with a known chronic disease, clinical complaints of Vitamin D deficiency or excess, abnormal physical examination findings, those who have used depot or daily doses of Vitamin D preparations within the last 3 months, and those who have lived in another region within the last 6 months were excluded from the study. Ca⁺², P, ALP, Mg and PTH levels were examined simultaneously with vitamin D levels.

After the biochemical parameters were examined, the remaining serum samples were stored in Eppendorf tubes in a -20°C deep freezer until genetic analysis. DNA was isolated from stored samples and ApaI and TaqI gen polymorphisms were studied (Figure 1, 2).

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 20 was used for the analysis of the data. Two groups were formed in terms of Vitamin D level: Group 1 included children with Vitamin D < 20 ng/mL and group 2 included those with Vitamin D ≥ 20 ng/mL. Visual graphics (Histogram) and the Kolmogorov-Smirnov test were used to check whether parameters complied with normal distribution. The relationship between the genotype and allele distributions of TaqI and ApaI polymorphisms and Vitamin D levels of the two groups was evaluated by Pearson's Chi-Square test. The vitamin D results of the groups with ApaI and TaqI polymorphisms were evaluated with the ANOVA test. P<0.05 values were considered statistically significant in all tests.

Results

The mean age of 124 healthy children participating in the study was 8.11 (4.98) years. Groups 1 and 2 (9.38 (4.87) years vs. 6.38 (4.62) years) and females and males (8.47 (5.33) years vs. 7.86 (4.74) years) were similar in terms of age (P=0.091 and P=0.603, respectively). There were 36 females and 36 males in Group 1, and 14 females and 38 males in Group 2.

Figure 1: A: Cutting with ApaI among children with Vitamin D < 20ng/mL, B: Cutting with ApaI among children with Vitamin D ≥20ng/ml

Figure 2: A: Cutting with TaqI among children with Vitamin D < 20ng/mL, B: Cutting with TaqI among children with Vitamin D ≥ 20 ng/mL
The mean vitamin D levels in Groups 1, 2 and overall were 13.82 (3.29) ng/mL, 33.96 (20.47) ng/mL, and 23.49 (21.54) ng/mL, respectively, with no significant difference between the groups (P = 0.509). The lowest and highest vitamin D level among all children were 5 ng/mL and 116 ng/mL, respectively. The mean vitamin D levels of male and female children were 24.73 (17.64) ng/mL and 18.62 (14.69) ng/mL, respectively (P < 0.001) (Table 1).

In terms of Apal polymorphism, 32% had AA genotype, 57% had Aa genotype, and 11% had aa genotype. For TaqI polymorphism, 42% had TT genotype, 48% had Tt genotype, 10% had tt genotype (Table 2).

According to studies conducted in different age groups of many societies, Vitamin D insufficiency and deficiency are at a remarkable level all over the world [10-13]. Research in Turkey showed that rates of overall vitamin D deficiency and insufficiency were 51.8% and 20.7%, respectively. The mean vitamin D level in deficient individuals was 18.7 (6.8) ng/mL in Erzurum, and 52% of children aged between 2-4 years, 62.5% of children aged between 5-8 years and 63% of children aged between 9-12 years were vitamin D deficient [14,15].

In the present study, mean Vitamin D levels were 23.49 ng/mL. Among 124 children, Vitamin D levels were below 20 ng/mL in 72 (58%) cases and within normal limits in 52 (42%). None had severe deficiency or intoxication, while vitamin D levels of 34.7% were deficient, 23.4% were insufficient, 41.4% were normal, and 0.8% were excess (Table 4). In this respect, this study was compatible with other studies conducted in our region in terms of Vitamin D deficiency/insufficiency.

Table 1: Mean 25(OH)D3 across all population and genders

| Group | 25(OH)D3 Average | n | P-value |
|-------|------------------|---|---------|
| Vitamin D <20 ng/mL | 13.82 (3.29) | 72 | 0.58 |
| Vitamin D ≥20ng/mL | 33.96 (20.47) | 52 | 0.509 |
| All Population | 24.73 (17.64) | 124 (100%) | 0.001 |

Table 2: Distribution of Apal, TaqI polymorphism genotypes among the overall population

| Vitamin D <20ng/mL | Vitamin D ≥20ng/mL | All Population | Total | Boy | P-value |
|---------------------|---------------------|----------------|-------|-----|---------|
| (n=72)              | (n=52)              | (n=124)        | (n=50) | (n=74) |         |
| Apal                |                     |                |       |     |         |
| AA                  | 25 (35%)            | 40             | 32    | 18  | 0.656  |
| Aa                  | 40 (55%)            | 71             | 57    | 28  | 0.001  |
| aa                  | 10 (14%)            | 13             | 11    | 4   |         |
| TaqI                |                     |                |       |     |         |
| TT                  | 31 (43%)            | 52             | 42    | 19  | 0.175  |
| Tt                  | 34 (47%)            | 59             | 48    | 28  | 0.348  |
| tt                  | 7 (10%)             | 13             | 10    | 3   |         |

Table 3: Relationship between Vitamin D level and Apal and TaqI polymorphisms genotypes

| Vitamin D <20ng/mL | Vitamin D ≥20ng/mL | TT | Tt | Tt | P-value |
|--------------------|---------------------|----|----|----|---------|
| (n=124)            | (n=11)              | 24.73 (17.64) | 20.23 (12.51) | 0.509 |

Discussion

According to studies conducted in different age groups of many societies, Vitamin D insufficiency and deficiency are at a remarkable level all over the world [10-13]. Research in Turkey showed that rates of overall vitamin D deficiency and insufficiency were 51.8% and 20.7%, respectively. The mean vitamin D level in deficient individuals was 18.7 (6.8) ng/mL in Erzurum, and 52% of children aged between 2-4 years, 62.5% of children aged between 5-8 years and 63% of children aged between 9-12 years were vitamin D deficient [14,15].

In the present study, mean Vitamin D levels were 23.49 ng/mL. Among 124 children, Vitamin D levels were below 20 ng/mL in 72 (58%) cases and within normal limits in 52 (42%). None had severe deficiency or intoxication, while vitamin D levels of 34.7% were deficient, 23.4% were insufficient, 41.4% were normal, and 0.8% were excess (Table 4). In this respect, this study was compatible with other studies conducted in our region in terms of Vitamin D deficiency/insufficiency.
This study demonstrated that Apal and TaqI VDR gene polymorphisms do not pose a risk for Vitamin D deficiency and insufficiency, similar to studies that did not detect a relationship between VDR gene polymorphisms and vitamin D levels in the literature.

Limitations
Although VDR has different genetic polymorphisms, this study was conducted with two. The failure to include other polymorphisms can be considered as the limitation of this study.

Conclusion
Vitamin D deficiency is still an important societal problem today. The study group only includes healthy children and has a wide age range (12 months-18 years). The study population is sufficient compared to other studies, and it is the first VDR gene polymorphism study conducted in the relevant region. In light of all these data, it is thought that the study will contribute to further studies to be conducted in this regard.

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References
1. Gupta A, Thompson PD. The relationship of Vitamin D deficiency to statin myopathy. Atherosclerosis. 2011;215(1):23-9.
2. Gürel G, Karadol M, Çağrı E. A case-control study on rosacea and vitamin D levels. J Surg Med. 2018;2(3):269-72.
3. Helek MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiocascular disease. Am J Clin Nutr. 2004;80:1678-88.
4. Valdiviezo J.M, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clin Chim Acta. 2006;371(1-2):1-12.
5. Junaid K, Rehman A, Jaffar H, Ali S. Vitamin D receptor gene polymorphism in Indian population and its association with 25-hydroxy Vitamin D levels. Indian J Hum Genet. 2009;15(3):108–13.
6. Smolders J, Damoiseaux J, Menheere P, Tervaert JW, Hupperts R. FokI and TaqI VDR gene polymorphisms in major depression. Psychiatria Danubina. 2017;29(2):179-85.
7. Yıldız Ü, Ünlüvar E, Zeybek Ü, Topçu B, Cüce A, Toprak S, et al. Çocuklarda kliniğinde klinik Vitamin D düzeyleri. J Immunol Res. 2015;2015:646-50.
8. Yıldız Ü, Ünlüvar E, Zeybek Ü, Topçu B, Cüce A, Toprak S, et al. Çocuklarak biomecanik testlerde klinik Vitamin D düzeyleri. J Immunol Res. 2015;2015:646-50.