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Original Article

Influence of Serum Vitamin D Levels and Vitamin D Receptor (VDR) Polymorphisms on Susceptibility to Dental Caries

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ABSTRACT

Proper mineralization of teeth, bones, and other calcified tissues relies on vitamin D, which regulates calcium and phosphate homeostasis. The vitamin D receptor (VDR) gene must be activated for vitamin D to exert its effects. This research aimed to explore the influence of serum vitamin D levels and VDR gene variations on the development of dental caries in children. A total of 128 children aged 3–6 years participated, split evenly into two groups: 64 caries-free and 64 with dental caries. Blood samples were collected to quantify serum 25-hydroxyvitamin D concentrations. Oral examinations were performed to determine dmft index scores. For genetic assessment, 26 children (13 from each group) underwent PCR amplification and Sanger sequencing to detect variations in the VDR gene. Data analysis included chi-square or trend tests for categorical variables, Student's t-test or Mann–Whitney U test for continuous variables depending on normality, and Kruskal–Wallis tests for comparisons among three or more non-normally distributed groups. Children with caries generally had lower serum vitamin D levels than their caries-free counterparts, but this difference did not reach statistical significance. No mutations in the VDR gene were observed in either group. Furthermore, the frequency of ApaI, TaqI, and FokI polymorphisms was similar between groups. These outcomes indicate that routine vitamin D supplementation may not be effective as a preventive strategy against dental caries. The study sheds light on the interplay between vitamin D, VDR genetic variants, and dental caries in preschool-aged children. These findings contribute to understanding potential risk factors for oral health and may guide the design of future preventive measures, though additional research is required to clarify the role of vitamin D.

Keywords: Dental caries, Vitamin D deficiency, 25-Hydroxyvitamin D, VDR gene polymorphisms

Introduction

Maintaining oral health is a key public health concern, as it significantly impacts overall well-being and quality of life. Among oral conditions, dental caries remains one of the most prevalent problems affecting children and adults alike [1, 2]. This chronic, infectious disease arises from a combination of microbial, behavioral, genetic, and environmental factors [2, 3].

Vitamin D, classified as a prohormone, exerts effects through endocrine, autocrine, and paracrine mechanisms. It is vital for calcium (Ca^{2+}) homeostasis and skeletal metabolism, while also enhancing the uptake and use of calcium and phosphorus (Ca^{2+}–P^{3-}), which contributes to mineral deposition in bones and teeth [4, 5]. In addition to its skeletal roles, vitamin D has been implicated in modulating immune function, cognitive performance, blood pressure, cardiometabolic health, and aging-related processes [4, 6].

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Regulation of serum Ca^{2+}, P^{3-}, and alkaline phosphatase (ALP) is largely dependent on vitamin D. By maintaining appropriate levels, vitamin D supports both bone remodeling and calcium-phosphate equilibrium [7, 8]. Routine clinical assessments frequently include measurements of ALP, Ca^{2+}, and P^{3-}, alongside hemoglobin (HMG), which is responsible for oxygen transport in the blood [9, 10].

Among available biomarkers, circulating 25-hydroxyvitamin D (25(OH)D, calcidiol) serves as the most reliable indicator of total vitamin D from dietary intake and endogenous production [11]. Serum 25(OH)D measurement is preferred for detecting deficiency due to its relatively long half-life of approximately three weeks and higher plasma concentration compared with the biologically active form, 1,25-dihydroxy vitamin D ($1,25(OH)_2D$, calcitriol) [12].

The biological activity of vitamin D is mediated through its binding to the intracellular vitamin D receptor (VDR). Genetic variations in the VDR gene can modify receptor structure and function, influencing calcium metabolism, cellular proliferation, and associated regulatory pathways [13].

While multiple studies have linked vitamin D deficiency to impaired tooth mineralization, results are not always consistent. Evidence suggests that increased serum 25(OH)D levels achieved via supplementation may lower the risk of dental caries [4, 14–17].

Despite extensive research on the general health implications of vitamin D, there remains a paucity of studies specifically examining its direct effects on dental caries.

Materials and Methods

Study design and participants

The study protocol followed the ethical principles described in the World Medical Association Declaration of Helsinki. Prior to participation, written informed consent was obtained from each child's parent or legal guardian. The investigation took place at Ege University Faculty of Medicine Children's Hospital between December 2021 and March 2022.

Determination of sample size

To establish the number of participants needed for biochemical analyses, a power calculation was conducted by the Department of Biostatistics and Medical Informatics at Ege University. Comparisons of serum 25-hydroxyvitamin D (25(OH)D) concentrations between children with dental caries and those without were planned using an independent samples t-test. Considering a medium effect size (d = 0.5), a significance level of 0.05, and statistical power of 80%, it was determined that each group should include 64 children.

A total of 128 children, aged 3 to 6 years, were enrolled. All participants were systemically healthy, had no physical impairments, and had not undergone blood tests within the past year. Among them, 64 children had at least one carious lesion, whereas the remaining 64 were caries-free. Recruitment occurred through the Child Health and Diseases Department and the Dentistry Clinic of Ege University Children's Hospital. Participants were selected randomly, and the distribution of males and females was balanced between groups.

Children were excluded if they had chronic conditions influencing vitamin D metabolism, including obesity, type 1 diabetes, celiac disease, asthma, or hypertension. Additional exclusion criteria included endocrine, cardiovascular, or renal disorders such as Turner syndrome, hypothyroidism, or renal insufficiency; ages outside 18 to 72 months; congenital dental anomalies; or cognitive or developmental disabilities.

For the genetic analysis component, blood samples were collected from children whose parents agreed to participate. Comprehensive examination of the vitamin D receptor (VDR) gene was performed on 26 children, with an equal number from the caries and caries-free groups (13 each).

Data collection

Oral examination and survey

Each participant underwent a thorough oral evaluation at the Dentistry Clinic of Ege University Children's Hospital. To maintain consistency, a single investigator conducted all examinations using an intraoral mirror and probe under reflector lighting. The dmft index was calculated according to the 1997 guidelines provided by the World Health Organization.

Parents completed a structured survey designed to collect information on their child's medical history, dietary patterns, awareness of vitamin D, oral hygiene routines, and socio-economic background. Questions covered daily sugar intake, history of professional fluoride treatments, and routine use of fluoride-containing toothpaste.

Consumption of vitamin D-rich animal products—milk, cheese, eggs, and fish—was also recorded. Responses were classified into three categories: children consuming at least one of these foods daily, those consuming fewer than two, and those who rarely ate these products. The survey additionally asked about the daily intake of vitamin D supplements or multivitamins containing vitamin D. All surveys were completed by the parents of participants in both groups.

Biochemical assessment

Venous blood samples were drawn from all 128 participants to perform biochemical analyses. Three separate tubes were used for different assays, and standard laboratory protocols were followed to determine serum calcium (Ca^{2+}), phosphate (P^{3-}), alkaline phosphatase (ALP), 25-hydroxyvitamin D [25(OH)D], and complete blood count (CBC) parameters.

- Five milliliters of blood for Ca^{2+}, ALP, and P^{3-} analysis were collected in yellow-top tubes containing a serum-separating gel.
- Two milliliters for CBC measurement were collected in purple-top K_2-EDTA tubes, with hemoglobin (HGB) levels extracted from the CBC results.
- Two milliliters for vitamin D measurement were drawn into purple-top K_2-EDTA tubes.

Serum 25(OH)D levels were quantified using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with an Agilent 6460 Triple Quadrupole system (Agilent Technologies, Santa Clara, CA, USA).

Due to variations in vitamin D reference ranges in previous literature, this study incorporated commonly cited cutoffs, including optimal and suboptimal levels, alongside laboratory-specific reference values, allowing a comprehensive assessment. Since no significant discrepancies were observed, the reference values were consolidated. Serum 25(OH)D concentrations were reported according to two separate reference standards, as summarized in **Table 1**.

Table 1. Reference ranges for serum vitamin D concentrations [11, 14, 18–20].

Baseline Reference Range 1		**Ege University Faculty of Medicine Reference Range 2**	
Condition	**Serum 25(OH)D Level**	**Condition**	**Serum 25(OH)D Level**
Severe Shortage	0–9 ng/mL	Major Deficiency	≤10 ng/mL
Insufficient	10–19 ng/mL	Slight to Moderate Deficiency	10–19 ng/mL
Below Optimal	20–29 ng/mL	Ideal Range	20–50 ng/mL
Ideal	30–49 ng/mL	Elevated Risk for Hypercalciuria	51–80 ng/mL
Elevated	≥50 ng/mL	Potential Toxicity	≥80 ng/mL

Genetic analysis

For the genetic component of the study, blood samples were collected only from children whose parents had given consent for molecular testing. A total of 26 participants were included in this analysis, with 13 children randomly selected from each group to ensure equal representation.

To examine mutations and polymorphisms in the VDR gene, one venous blood tube was drawn per participant. Following pediatric clinical trial ethical guidelines, blood collection did not exceed 0.8 mL per kilogram of body weight at a single time point [21]. Two milliliters of blood for genetic testing were collected in K_2-EDTA purple-top tubes and sent to the Molecular Genetics Laboratory at the Medical Genetics Department. VDR gene variants were analyzed using polymerase chain reaction (PCR) followed by visualization with agarose gel electrophoresis. The sequencing strategy targeted all coding exons and exon–intron junctions of the VDR gene. Genomic DNA was isolated from blood cells using the QIAcube Connect platform in combination with the QIAcube AllPrep DNA/RNA FFPE kit (Product No: 80234, QIAGEN, Hilden, Germany). DNA yield and purity were assessed spectrophotometrically with a ThermoScientific NanoDrop™ One/OneC (Thermo Fisher Scientific, Waltham, MA, USA).

Amplification of VDR exons was carried out using primers specified in **Table 2**. The PCR products underwent dye-termination sequencing with a DNA sequencing kit (Perkin-Elmer, Foster, CA, USA) and were analyzed on

the ABI Prism 3100 sequencer (Applied Biosystems, Foster, CA, USA). Identified gene variations were interpreted based on data from the Ensembl genome database and the Human Gene Mutation Database (HGMD).

Table 2. Primer sequences used to amplify VDR coding exons and exon–intron junctions.

Primers		Sequence (5′-3′)
3	forward	GCACCAAGGATGCCAGC
	reverse	CCTTCATGGAAACACCTTGC
4	forward	GTGATGACAGGGTGAGGAGC
	reverse	AAGGCCTTTCCCTGACTCC
5	forward	AAGGTTTCCTGGAGGAGCTG
	reverse	CCCTCTGTCCCTACTCCCTG
6	forward	ATCAGGGCCAAGGTAGGAAG
	reverse	GTGCGGTGGACTCCTCG
7	forward	CAGAGGGAAGCCTGGGGCT
	reverse	GTGGTGGATGAGTGATCTCCAACCC
8/9	forward	TGATTTGTGTGGCTTGAAGG
	reverse	TTTGTCCTTCATACTCCCCG
10	forward	GGTGGTGGGATTGAGCAG
	reverse	ACGTGGCCCTGGAGGAG

Statistical analysis

Data from biochemical assays and genetic evaluations were processed with SPSS 25.0 (IBM, New York, NY, USA). The Kolmogorov–Smirnov test was applied to determine whether variables followed a normal distribution. Categorical variables were compared across groups using chi-square tests and chi-square tests for trend. For two-group comparisons, parametric (Student's t-test) or nonparametric (Mann–Whitney U) tests were selected based on data distribution. Comparisons involving three or more groups employed the Kruskal–Wallis test. Since no statistically significant differences were detected, no post hoc testing was performed. A significance threshold of 0.05 was applied for all analyses.

The relationship between specific VDR gene polymorphisms and the presence of dental caries was assessed using the chi-square test of independence.

Results and Discussion

The study population consisted of 128 children aged 3–6 years who attended either the Department of Child Health and Diseases or the Dentistry Clinic at Ege University Children's Hospital between December 17, 2021, and March 3, 2022, for routine evaluation or treatment. The cohort was evenly split by sex (64 males, 64 females), and the median age was 5 years, ranging from 3 to 6 years. **Table 3** provides a summary of the participants' demographic and clinical characteristics. The mean age across all participants was 4.96 ± 1.068 years (mean ± standard deviation).

Table 3. Demographic and clinical characteristics of the enrolled children.

Variable	Category	Caries-free (n = 64) n (%)	Caries (n = 64) n (%)	Test Statistic	p-value
Age (years)	3	14 (21.9)	2 (3.1)	Chi-square trend = 28.949*	0.000
	4	19 (29.7)	8 (12.5)		
	5	19 (29.7)	12 (18.8)		
	6	12 (18.8)	42 (65.6)		
Sex	Female	32 (50.0)	32 (50.0)	Chi-square = 0.000**	1.000

	Male	32 (50.0)	32 (50.0)		
Tooth brushing frequency (times/day)	Less than 2	44 (68.7)	45 (71.3)	Chi-square = 1.368	0.505
	2 or more	20 (31.3)	19 (29.7)		
Person responsible for brushing	Child alone	32 (50.0)	35 (54.7)	Chi-square = 0.407	0.816
	Child with family	23 (35.9)	22 (34.4)		
	Family only	9 (14.1)	7 (10.9)		
Routine dental check-ups (every 6 months)	Yes	1 (1.6)	7 (10.9)	Chi-square = 4.879	0.087
	Longer than 6 months	10 (15.6)	8 (12.5)		
	No	53 (82.8)	49 (76.6)		
Fluoride therapy in past 6 months	Yes	1 (1.6)	1 (1.6)	Chi-square = 0.000	1.000
	No	63 (98.4)	63 (98.4)		
Use of fluoridated toothpaste	Yes	22 (34.4)	45 (70.3)	Chi-square = 16.568	0.000
	No	42 (65.6)	19 (29.7)		
Sugar intake between meals	Never	8 (12.5)	1 (1.6)	Chi-square = 8.098	0.017
	Rarely	29 (45.3)	24 (37.5)		
	Daily	27 (42.2)	39 (60.9)		
Serum vitamin D measurement	Yes	15 (23.4)	13 (20.3)	Chi-square = 0.183	0.669
	No	49 (76.6)	51 (79.7)		
Vitamin D supplementation	Yes	23 (35.9)	13 (20.3)	Chi-square = 3.865	0.049
	No	41 (64.1)	51 (79.7)		
Daily consumption of milk, cheese, eggs, fish	Does not eat	8 (12.5)	5 (7.8)	Chi-square = 5.824	0.054
	Rarely	4 (6.3)	13 (20.3)		
	At least one item daily	52 (81.3)	46 (71.9)		

All results are expressed as averages with 95% confidence intervals. For categorical comparisons, significance levels (p values) were derived from complex-sample chi-square trend assessments (*), while overall group comparisons used chi-square tests (**).

Among the 128 participants, the dmft index demonstrated an overall mean of 2.79 ± 3.46, with a median of 0.50 (minimum 0.00; maximum 12.0). Caries frequency was observed to rise progressively with age, and this age–caries association was statistically significant ($p = 0.000$).

The outcomes of the biochemical analyses are summarized in **Table 4**, which outlines the distribution of 25(OH)D levels and their relationship with dental caries. Children whose serum 25(OH)D concentration fell below 30 ng/mL showed a greater prevalence of caries than those with concentrations of 30 ng/mL or above. In the three-level classification system, individuals with severe deficiency (0–9 ng/mL) presented a slightly higher rate of caries compared with those with optimal vitamin D status (20–50 ng/mL); however, this difference lacked statistical significance.

Average 25(OH)D levels were 20.41 ng/mL (range: 3.00–50.00) in the caries-free cohort and 17.97 ng/mL (range: 5.00–43.00) in children with caries. Statistical testing confirmed that vitamin D concentrations were not significantly associated with dental caries ($p = 0.642$).

Table 4. Serum profiles of 25(OH)D, hemoglobin (HGB), calcium (Ca^{2+}), phosphate (P^{3-}), and alkaline phosphatase (ALP) in relation to caries status.

Variable	Category	Caries-free (n = 64) n (%)	Caries (n = 64) n (%)	Test statistic	p-value
25(OH)D (two-level grouping)	Insufficient/Below optimal (3–29 ng/mL)	50 (78.1)	55 (85.9)	$\chi^2 = 1.325$	0.250
	Adequate range (30–50 ng/mL)	14 (21.9)	9 (14.1)		
25(OH)D (three-level grouping)	Severe deficit (0–9 ng/mL)	7 (5.5)	9 (7.0)	$\chi^2 = 0.888$	0.642
	Moderate deficit (10–19 ng/mL)	29 (22.7)	32 (25.0)		
	Optimal status (20–50 ng/mL)	28 (21.9)	23 (18.0)		
Hemoglobin (HGB)	Below reference (<11.5 g/dL)	15 (23.4)	14 (22.2)	$\chi^2 = 0.027$	0.870
	Within reference (11.5–14.5 g/dL)	49 (76.6)	49 (77.8)		
Calcium (Ca^{2+})	Normal (8.6–10.2 mg/dL)	57 (89.1)	62 (96.9)	$\chi^2 = 2.988$	0.084
	Elevated (>10.2 mg/dL)	7 (10.9)	2 (3.1)		
Phosphate (P^{3-})	Normal (3.1–6 mg/dL)	64 (100.0)	63 (98.4)	$\chi^2 = 1.008$	0.315
	Elevated (>6 mg/dL)	0 (0.0)	1 (1.6)		
Alkaline phosphatase (ALP)	Below normal (<142 IU/mL)	5 (7.8)	5 (7.8)	$\chi^2 = 1.009$	0.604
	Normal range (142–335 IU/mL)	59 (92.2)	58 (90.6)		
	Above normal (>335 IU/mL)	0 (0.0)	1 (1.6)		

The children included in this study were categorized according to their age, and Fisher's exact test was applied to assess the association between age and caries occurrence. No statistically meaningful differences were observed across age categories (3 years: p = 0.342; 4 years: p = 0.669; 5 years: p = 0.644; 6 years: p = 0.920).

Table 5 summarizes the correlation results between serum vitamin D concentrations and other biochemical indicators. Correlation analyses conducted between vitamin D and hemoglobin (HGB), phosphate (P−3), alkaline phosphatase (ALP), and calcium (Ca^{2+}) revealed no significant associations. Similarly, analysis of the relationship among age, dmft index values, and vitamin D showed no statistical correlation.

Table 5. Associations between vitamin D and biochemical/clinical variables.

Variable	Correlation coefficient (Spearman's rho)	p-value
dmft	−0.151	**p-value**
Age	−0.127	0.088
HGB	−0.075	0.153
Ca^{2+}	0.115	0.403
P^{-3}	−0.088	0.196
ALP	−0.119	0.322

In the genetic part of the research, Sanger sequencing was performed on 13 children without caries and 13 with caries. The analysis covered exons 3 through 10 as well as exon–intron boundary regions. Apart from previously described polymorphisms, no novel variants were identified **(Table 6)**.

Table 6. Distribution of polymorphisms in caries-free versus caries-affected groups (NM_000376.3 transcript reference).

Variable	Correlation coefficient (Spearman's rho)	p-value
dmft	−0.151	**p-value**
Age	−0.127	0.088

HGB	−0.075	0.153
Ca^{2+}	0.115	0.403
P^{-3}	−0.088	0.196
ALP	−0.119	0.322

- In the third analysis step, data were obtained from 12 children without dental caries.

A total of 25 participants were examined for the third exon, where the FokI variant was identified in 11 children from the caries-free group and 12 children with caries. For the tenth exon, sequencing was conducted in 26 participants, and the TaqI variant was found in 10 individuals from each group. The tenth exon together with its exon–intron boundary was also evaluated in 26 children, with the ApaI polymorphism being observed in 12 children free of caries and 11 with caries.

Figure 1 provides representative outputs from sequence analysis. In the chromatograms, adenine is shown in green, guanine in black, and cytosine in blue. When the peaks align perfectly with the reference genome, it indicates absence of variation. The appearance of two peaks in different colors at the same location reflects a heterozygous change, while distinct peaks in both alleles that differ from the reference sequence represent a homozygous alteration.

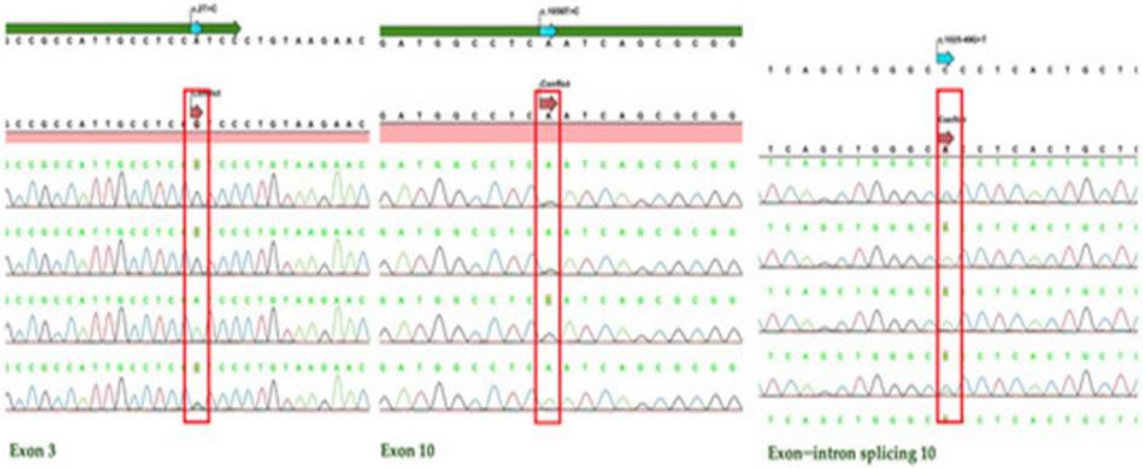

Figure 1. Representative chromatograms displaying polymorphisms in exons and exon–intron regions.

The present investigation examined how both biochemical indicators and questionnaire findings relate to the occurrence of dental caries, with emphasis on serum vitamin D status and VDR gene polymorphisms. The outcomes were subsequently compared with reports available in earlier literature.

The metabolic behavior of vitamin D is noteworthy: the active metabolite, $1,25(OH)_2D$, is cleared from circulation within 3–6 hours, while the storage form, 25(OH)D, remains measurable for roughly three weeks. Concentrations are expressed differently—pg/mL for $1,25(OH)_2D$ and ng/mL for 25(OH)D [22]. In this project, the longer-lasting form, 25(OH)D, was quantified using liquid chromatography–tandem mass spectrometry, a method chosen for its sensitivity.

Since no global consensus exists on the threshold for sufficient vitamin D, different cut-off points are employed in prior research [15, 19, 23–26]. Commonly, ≥30 ng/mL is treated as adequate, 20–30 ng/mL as insufficient, 10–20 ng/mL as deficient, and levels <10 ng/mL as severely deficient [14, 15, 27]. These intervals served as the primary interpretive framework in our analysis.

For comparison, we also incorporated the ranges currently adopted by the Faculty of Medicine at Ege University [16, 28]. Outcomes were not statistically different when applying these two alternative schemes.

Across the cohort, 12.5% of participants exhibited severe deficiency, and a combined 82% showed either deficiency or insufficiency. The average concentration of serum vitamin D was 19.19 ng/mL. Other biochemical markers remained largely within expected physiological intervals when assessed alongside vitamin D status. The high frequency of vitamin D deficiency observed here is consistent with findings from other national surveys [9, 29].

Vitamin D contributes fundamentally to skeletal mineralization and is involved from the earliest phases of jaw and tooth development. Beyond odontogenesis, it is thought to continue influencing the occurrence of dental caries [30].

The connection between vitamin D and the calcium–phosphorus system has long been established. Its deficiency is a primary cause of rickets in childhood, a condition that impairs normal bone growth and frequently results in oral complications [31–33]. Research also notes that children suffering from early caries often display reduced calcium levels in serum [34]. Our results, however, did not identify a measurable relationship between vitamin D concentration and Ca^{2+} or P^{3-} levels.

In this investigation, 128 healthy children aged 3–6 years were studied. Data showed that the dmft index rose steadily with age. This observation aligns with previous reports [35–37] and is most likely due to longer exposure of erupted teeth to cariogenic conditions the older the child becomes.

Some publications have highlighted that vitamin D insufficiency (<20 ng/mL) increases susceptibility to caries [16, 23]. Others, which adopt 30 ng/mL as the adequate threshold, suggest vitamin D functions as a protective factor [11, 25, 34]. On the other hand, a U.S. survey acknowledged a link but argued the results were inconclusive [26]. In our sample, 21.9% of caries-free children versus 18% of those with caries had sufficient vitamin D; the difference was not significant. When 30 ng/mL was used as the cutoff, 21.9% of unaffected children and 14.1% of caries-positive children reached optimal levels, again showing no statistical difference.

Literature outcomes remain inconsistent. For instance, one study associated vitamin D with permanent tooth decay but not with mixed dentition [24]. Dutch research described only a minor link between vitamin D deficiency and caries in the primary dentition period [38]. Among children with special needs, those below 20 ng/mL vitamin D presented double the caries rate, suggesting a stronger effect in this subgroup [39]. Conversely, several investigations failed to establish any meaningful association [28, 40–42].

Several meta-analyses have attempted to clarify the connection between vitamin D and dental caries, but the evidence remains inconclusive, and establishing a clear, statistically significant relationship has proven difficult [43]. One systematic review noted only a minor association between low vitamin D levels and oral disease, underscoring the importance of conducting long-term observational studies to resolve uncertainties [44]. In our cohort, serum vitamin D levels tended to inversely correspond with caries prevalence; however, this trend was not statistically meaningful, reflecting the complex interplay of factors involved in caries development.

Our study also examined genetic variations in the vitamin D receptor (VDR) gene. The bioactive hormone $1,25(OH)_2D_3$ exerts its physiological effects by binding to the nuclear receptor encoded by VDR, which is widely expressed across multiple tissues [45]. In many of these tissues, enzymes catalyze the conversion of circulating 25(OH)D into $1,25(OH)_2D$, enabling the hormone to act locally [12, 46].

The VDR receptor modulates the expression of target genes responsible for calcium and bone homeostasis [47]. Mutations in VDR may lead to reduced serum Ca^{2+} and phosphate, elevated parathyroid hormone (PTH) and alkaline phosphatase (ALP), and, in some cases, very high $1,25(OH)_2D$ levels [48, 49].

Beyond skeletal outcomes, mounting evidence implicates VDR function in chronic disease prevention, including cancer, autoimmune disorders, diabetes, infections, metabolic syndrome, and cardiovascular conditions [12, 46]. The VDR gene exhibits high polymorphism, with over 470 single-nucleotide polymorphisms (SNPs) identified to date [46]. Among these, the variants most frequently studied include FokI (rs2228570), BsmI (rs11544410), ApaI (rs7975232), and TaqI (rs731236) [50]. Investigations in the Turkish population have consistently reported FokI, ApaI, and TaqI as the most common polymorphisms [51]. Consistent with prior findings, our study detected FokI, TaqI, and ApaI variants within the examined regions of the VDR gene.

In 2021, a meta-analysis and systematic review evaluated the influence of several VDR gene polymorphisms — ApaI, FokI, TaqI, BsmI, and BglI — on dental caries. Among these, only the FokI variant demonstrated a statistically notable association with caries risk [52]. Another investigation also proposed a potential connection between FokI and dental caries [53]. In contrast, a separate study reported no detectable relationship between FokI or BglI polymorphisms, serum vitamin D concentrations, and dental caries [54]. Within our cohort, only 2 of 26 children lacked the FokI polymorphism, while ApaI appeared in both alleles in 52% of children and in a single allele in 40%. When comparing the caries-free and caries groups, the distribution of these variants was similar, and statistical analysis indicated no significant correlation with caries prevalence.

Further research has explored the impact of BsmI and ApaI variants on caries development [52]. Some literature indicates that BsmI may be linked to higher caries occurrence [55]. In the current study, ApaI was absent in 3 of 26 children, observed in one allele in 61.6%, and present in both alleles in 26.9%. Comparisons between the

caries-free and caries groups showed similar frequencies, and statistical tests did not reveal a significant association with dental caries.

Regarding the TaqI polymorphism, a meta-analysis reported a significant difference in its prevalence between children with and without caries [56]. Additional studies suggested a potential role of TaqI in dental caries, whereas other polymorphisms did not show a similar effect [13, 57]. Some authors hypothesize that even if TaqI does not directly determine caries incidence, the VDR gene may still influence the processes leading to caries formation [58]. Conversely, other studies have found no statistically significant link between TaqI and dental caries [59].

Strengths

Targeted age group: Participants were limited to children aged 3–6 years, corresponding to the primary dentition stage. This approach reduced developmental variability between teeth and helped ensure a more uniform sample.

Seasonal control: The study exclusively collected data during the winter months, minimizing the influence of seasonal changes on vitamin D levels and maintaining consistency across participants.

Ethnic uniformity: Conducting the research within a single region of Turkey allowed for a relatively homogeneous ethnic background, reducing confounding due to population differences. Future studies could expand to include multiple ethnic groups to investigate genetic variation more broadly.

Exclusion of confounding conditions: Children with chronic illnesses or physical disabilities that might affect oral health or biochemical measurements were not included, avoiding potential confounding effects.

Limitations

Cross-sectional design constraints: Because this study used a cross-sectional approach, causal relationships between vitamin D levels and dental caries cannot be established. Observed correlations are therefore less definitive than results from prospective studies.

Statistical power considerations: A post hoc analysis revealed power levels of 63% for one-tailed and 50% for two-tailed tests, suggesting that a larger cohort may be necessary to detect subtle effects.

Limited genetic sample: VDR gene analysis was performed using Sanger sequencing, which restricted the sample size for the genetic part of the study. Nevertheless, results confirmed the conserved nature of the VDR gene, as no mutations were identified in either caries-free or caries-affected children.

Potential for bias: Prior dental treatment or guidance among some participants could have influenced oral hygiene behaviors, which may have affected the study outcomes.

Conclusion

The findings from this study indicate that, among children aged 3–6 years, there is no statistically significant association between insufficient or deficient vitamin D levels and the prevalence of dental caries. While children without caries tended to have slightly higher serum vitamin D concentrations, this difference did not reach statistical significance. Analysis of the VDR gene did not reveal any mutations, and vitamin D levels showed no measurable correlation with other biochemical markers examined in the study.

Even though a clear link between vitamin D and dental caries was not established, the results underscore the value of educating parents on preventive oral hygiene measures. Future studies should aim for larger cohorts and more diverse populations, and investigate whether vitamin D supplementation could play a role in strategies designed to improve oral health and prevent dental caries.

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