Caries Risk Assessment in Children with Different Rates of Vitamin D Deficiency, Using Cariogram Model

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Abstract: *Objective*: Vitamin D plays a very important role in improving oral and dental health as well as general health. The present study aims to evaluate the risk of caries development risk of children with and without vitamin D deficiency using the Cariogram model.

Methods: This study included a total of 75 healthy children aged 6-12 years, of which 50 (35 girls and 15 boys) had different levels of vitamin D deficiency, and 25 (12 girls and 13 boys) had none. Their risk of developing new dental caries was assessed with Cariogram. SPSS v21.0 (IBM, USA) was used for analyzing the study data. In the statistical tests, a p-value less than 0.05 was considered statistically significant.

Results: Mean chronological and dental ages of the participants were obtained as 9±2.32 and 8±2.36, respectively. The distribution of salivary flow rate, buffering capacities, and distributions of Lactobacilli and S. mutans counts between the groups were found to be similar. There was a significant difference between Group I and Group II and between Group I and Group III in regard to the Cariogram green percentage (percentage of chances to avoid caries), p=0.002 and p<0.001, respectively.

Conclusion: In the present study, we observed a decrease in the Cariogram green sector percentage with low levels of vitamin D and an increase with normal vitamin D levels. Therefore, chances to avoid new dental caries were increased in sufficient levels of vitamin D.

Keywords: Dental caries risk, vitamin D, Cariogram.

INTRODUCTION

Besides dietary habits, microorganisms, oral hygiene status, and medication use, deficient intake of essential nutrients and vitamins are also effective in developing dental caries [1]. When there is an imbalance between nutrition /energy intake and body expenditure, malnutrition develops due to consequent vitamin, mineral, and protein deficiencies. Vitamin and mineral deficiencies associated with malnutrition may lead to dental hypoplasia, which in turn presents a risk for developing dental caries [2, 3]. Among those vitamin deficiencies, the correlation between low vitamin D levels and dental decay has been shown in some studies [4-7]. Vitamin D plays a key role in promoting general health as well as oral and dental health. This role starts with the developmental stages of the jaw and tooth and continues to affect dental caries after the tooth formation has been completed [8, 9]. An average of one billion people have been affected worldwide by vitamin D deficiency, which has a crucial role in calcium and phosphorus absorption [10, 11]. A recent study conducted in Erzurum province, Turkey, showed that vitamin D deficiency rickets (VDDR) was seen in 6.09 % of 0 to 3 -year-old children with very low levels of Ca, P, and ALP [12].

Identifying high-caries risk children is very important for effectively implementing preventative dental measures. Thus, caries risk prediction models (CRPMs) interactively assess the multiple caries-related risk factors recently developed [13-18]. Among those, Cariogram, an algorithm-based assessment program, is the most frequently used model [13, 14].

Cariogram establishes caries risk factors for the individuals and provides dentists with preventive and therapeutic strategies. It is a graphing software that helps interpret biological data and displays a possible caries risk situation. The software consists of an algorithm presenting the input data, mostly the analysis of biological factors, and also demonstrates the level of impact of different etiological factors on the caries risk factors [13].

Cariogram can simultaneously evaluate several caries-related factors and parameters such as caries experience, diet content and frequency, systemic diseases, level of fluoride intake, plaque index, Streptococcus mutans (S.mutans), and Lactobacillus counts, saliva flow rate and buffer capacity. Judgment of caries risk by Cariogram provides the advantage of

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recommending individualized preventive measures for the patients [13, 14].

Due to the risk of dental caries and hypoplasia associated with low Ca, P, and ALP levels seen in vitamin D deficient children, insufficient vitamin D levels commonly observed in local children, and an inadequate number of studies in literature, assessing caries-related risk factors in vitamin D deficiency, we aimed to evaluate the caries risk of the children living in Erzurum with different rates of vitamin D deficiency. The present study examined the impact of vitamin D deficiency on the risk factors in the development of dental caries by using Cariogram, unlike the classical Keyes-Jordan diagram, which also evaluated the systemic disorders such as vitamin D deficiency probably causing different individual symptoms.

MATERIALS AND METHOD

Study Design and Participants' Profile

This observational study was conducted between 2017-2019 and included a total of 75 children; 50 kids (35 girls and 15 boys) with no systemic diseases and with different rates of Vitamin D deficiency, and 25 healthy kids with no vitamin D deficiency and no previous treatment affecting their growth and development (12 girls and 13 boys).

Patient Selection Criteria

The study group included the children initially diagnosed with vitamin D deficiency and regularly followed up in the Department of Pediatrics at Ataturk University, Faculty of Medicine. The control group consisted of the healthy ones with normal vitamin D levels. Patients who agreed to participate in the study were referred to the Department of Pedodontics at Ataturk University, Faculty of Dentistry, where their oral & dental examinations were initially performed, and then saliva samples were collected. Written consent from the participating parents and child patients verbal assents was obtained.

Ethical Approval

The present study was initially designed for children with rickets and vitamin D deficiency, and approval of Ataturk University Faculty of Dentistry ethics committee # 71, session 11/2017 and dated 09/21/2017, was obtained. However, due to the inability to reach a sufficient number of children with rickets, it was decided to continue with vitamin D-deficient children. For this reason, ethical approval # 10/2018/84, dated 12.12.2018, was re-obtained from the Ataturk University Faculty of Dentistry ethics committee for the title and content change of the study.

Patient Inclusion Criteria

Inclusion criteria for control and study groups;

- Being between 6 and 12 years of age
- Having no systemic disease (Receiving no radiation and/or chemotherapy, diabetes, etc.)
- Receiving no hormone therapy
- No recent vitamin D supplementation
- Using no medications affecting saliva flow rate and buffering capacity
- Discontinuing any antibiotics treatment 10 days prior to participating in the study

Patients willing to participate in the study were informed about the procedures, medical tests, and examinations, and then families' written consents were obtained. Patients who applied to our clinic with extensive caries and pain and for routine check-ups and meeting the inclusion criteria were requested to have blood tests to measure their levels of vitamin D, and study groups were categorized in accordance with those determined vitamin D levels. Blood tests were done at Atatürk University, Department of Pediatrics. And a pediatrician evaluated the vitamin D levels.

Consequently, parents were interviewed face-to-face about their health status, and their answers were recorded. Obtained data were imported into the anamnesis forms.

Group I (G I): Patients with severe vitamin D deficiency (Vit. D level < 10 ng/ml)

Group II (G II): Patients with moderate vitamin D deficiency (Vit. D level ≥10-24 ng/ml)

Group III (G III): Patients with no vitamin D deficiency (optimal Vit. D level ≥25-80 ng/ml)

Dental Examination

Children’s dental examinations were performed in accordance with WHO guidelines, using a mouth mirror and probe.

DMFT /DMFS and df(t) /df(s) indices were determined. Silness-Löe plaque index was used to

assess oral hygiene, and the status of hypoplasia was recorded as "present" or "absent" [19].

Saliva Sampling and Analyses

S. Mutants and Lactobacillus Counts

Salivary S. mutants and Lactobacillus counts were determined with CRT® bacteria caries risk test (Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein). One side of the agar carrier was covered with S. mutans selective culture medium, and the other was covered with Lactobacillus selective culture medium.

After agar carriers were removed from the test vials and protective foils were peeled off from the two agar surfaces, both surfaces were thoroughly soaked with saliva using a special sterile pipette, and excess saliva was dripped off. A $NaHCO_3$-tablet was placed at the bottom of the test vial, and enough saliva was added to cover all vial surfaces fully. Then, agar carriers were re-inserted into the vials. The name of the patients and test dates were written on the tightly closed vials, and they were placed in an incubator (Ivoclar Vivadent AG, Schaan/Liechtenstein) not later than 10 min. after the culturing. They were incubated at 37^0 C for 48 hrs. Under appropriate lighting conditions, colony densities of S. mutans and lactobacillus on the surfaces of the agar carriers were evaluated and counted in accordance with the model chart enclosed in the kit.

Salivary Buffering Capacity

Buffering capacity was determined using CRT® Buffer test Kit (Ivoclar Vivadent AG, FL-9494 Schaan/Liechtenstein). Collected saliva was dropped onto the test field of the strip via a sterile pipette carefully without contacting the test field. Salivary buffering capacities were measured by comparing the color changes in the test strips with the sample color scale included in the buffer kit.

Salivary Flow Rate

The salivary flow rate was calculated using samples of stimulated saliva. For this purpose, parents were informed that their children should not have eaten anything but water for at least one hour before giving their saliva samples. Children were positioned comfortably on the dental chair, and they were requested to ingest their saliva after they chewed paraffin pellets for one minute. Then they were told to continue to chew the paraffin pellet for another five minutes and to spit into a cup. The secretion rate was expressed in mL/min.

Cariogram Assessment of Caries Risk Profile

Collected data were uploaded into Cariogram to estimate the participating children's caries-related risk profiles. Patients were evaluated in accordance with 5 Cariogram risk categories; "very high risk" = 0–20% "chance of avoiding caries"; "high risk" = 21–40% "chance of avoiding caries"; "moderate risk" = 41–60% "chance of avoiding caries"; "low risk" = 61–80% "chance of avoiding caries"; and "very low risk" =81–100% "chance of avoiding caries. Patients and their dentists were briefed about the calculated Cariogram risk category [13, 14].

Statistical Analysis

Statistical Package for the Social Sciences (SPSS v21) was used for analyzing the study data. Categorical variables were expressed as counts and percentages, whereas numerical variables were expressed as median, mean, standard deviation, and maximum and minimum values.

The normality of the numerical variables was assessed by the Kolmogorov-Smirnov test, Z-scores for skewness and kurtosis, and graphical methods. The Kruskal -Wallis test was used for comparing non-normally distributed numerical variables in the independent groups. The Bonferroni-corrected Mann-Whitney U test was used for the post hoc analyses. The distribution of the categorical variables in the independent groups was analyzed with the $\chi 2$ test. Association between the non-normally distributed numerical variables was measured with Spearman's Rho correlation analysis. $p<0.05$ was considered statistically significant.

RESULTS

Socio-Demographic Results

The distribution of 75 participating children (47 girls and 28 boys) by age and gender is given in Table **1**.

Of the participating children, 37.3 % were boys, and 62.7 % were girls, with a matching gender distribution in all groups except GI. The mean chronological age was 10± 2.07 in GI, 9±2.5 in GII, and 8±2.29 in GIII. The mean chronological age of the three groups was 9±2.32.

Biochemical and Microbiological Results

Saliva flow rate distributions in the groups are shown in Figure **1**. Flow rates by gender were found to be similar.

Table 1: Chronological Age and Gender Distribution of the Children by the Groups

Age	Gender	Study Groups (n=50)				Control Groups (n=25)	
		G I		GII		G III	
		n=25	Total	n=25	Total	n=25	Total
6-9	Female	4	5	8	11	6	15
	Male	1		3		9	
10-12	Female	15	20	8	14	6	10
	Male	5		6		4	

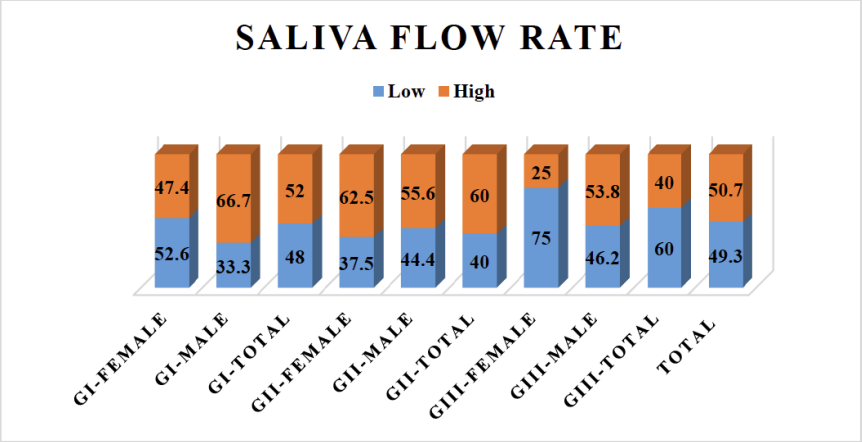

Figure 1: Saliva flow rate distribution in the groups.

The saliva buffer capacity of the groups was assessed only in terms of counts and percentages since cardiogram software boxes were not filled with sufficient data (Figure **2**). The highest percentage of buffer capacity in all three groups was the median buffer capacity, and it was determined as; 64%, 68%, and 64% respectively. The total lowest buffering capacity for all groups was measured as 17.3%.

The distribution of salivary lactobacillus in the groups is given in Figure **3**. Distributions of lactobacillus counts in all groups were similar. 15 children with higher counts of lactobacilli were found in GI (60%), 12 in GII (48%), and 14 in GIII (56%).

There was a significant difference in the distribution of lactobacillus counts by gender in GIII ($p<0.028$). In

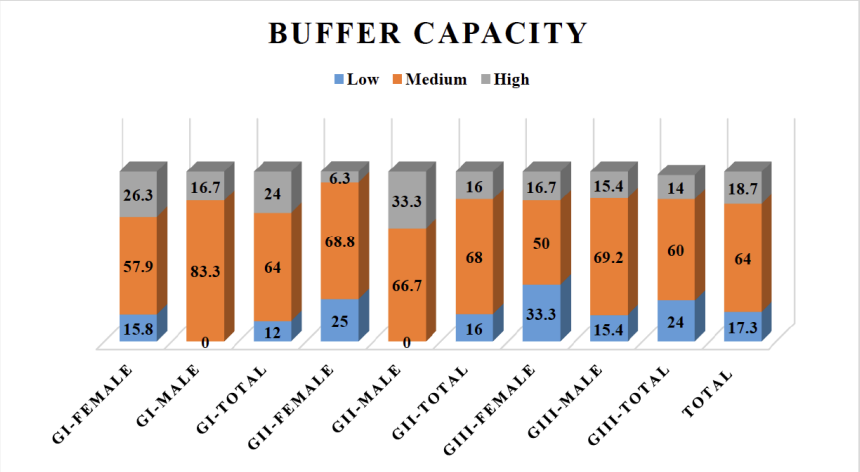

Figure 2: Distribution of saliva buffering capacity in the groups.

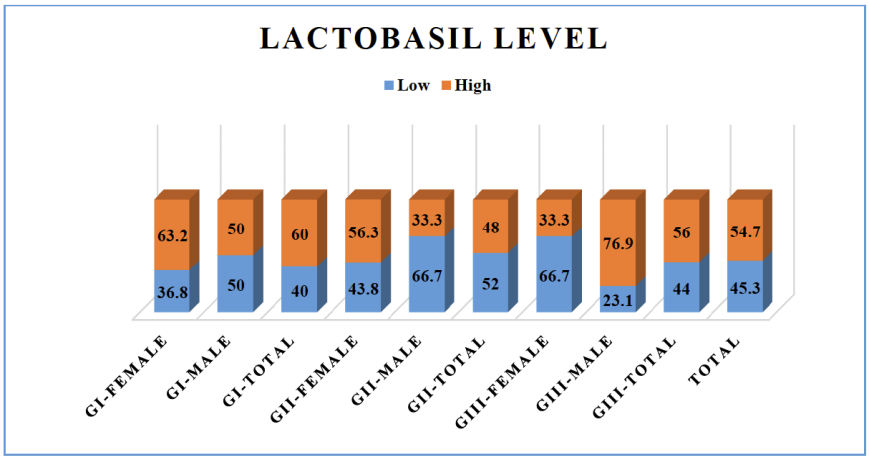

Figure 3: Lactobacillus counts in the groups.

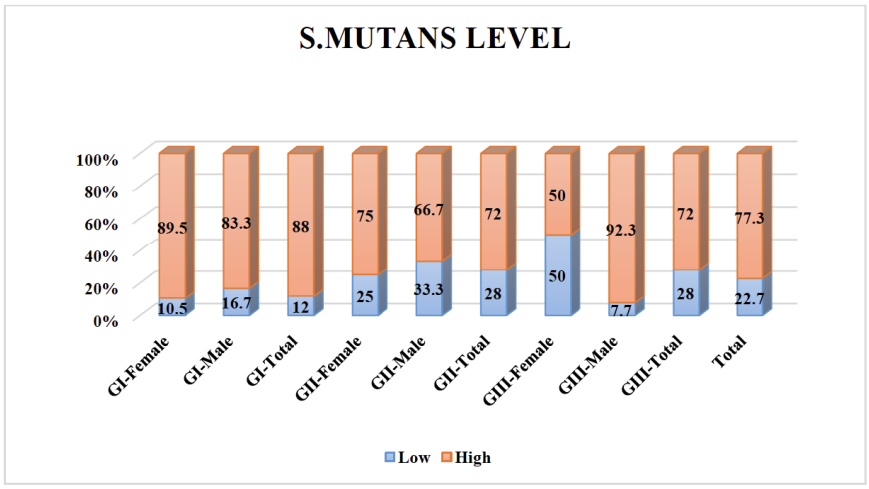

Figure 4: S. mutans count levels in the groups.

other groups, lactobacillus count distributions by gender were similar. With the exception of boys in GII and girls in GIII, mostly higher counts of lactobacillus were measured among other children.

The distribution of S.mutans counts is given in Figure **4**. S. mutans count distributions were matched in all groups. Significantly higher rates of S. mutans count distributions have been observed in the children in each of the three groups; 22 (88%), 18 (72%), and 18 (72%), respectively.

In another saying, there was an elevated caries activity in all groups. A significant difference has been observed among the distributions of lactobacillus counts by gender in GIII (p=0.03), which was caused by a higher S. mutans count rate seen in the boys (92.3%) and in the girls as well (50%). In the remaining groups, distributions of S. mutans counts were similar. However, in these groups, distributions of S. mutans counts were also measured in percentages.

Regarding the distribution of Cariogram green sector percentages, there were significant differences between GI-GII and GI-GIII (p=0.002, p<0.001, respectively). With respect to percentages of Cariogram green sector, obtained data showed a significant difference between vitamin D-deficient children in GI and others. Nevertheless, high levels of vitamin D were found to cause an increase in the percentage of Cariogram green sector (Table **2**).

The distribution of the Cariogram model for a chance of avoiding new caries is shown in Figure **5**. Since there was no sufficient data in the cardiogram software boxes for the variable of “avoiding new caries”, distribution was just assessed in terms of counts and percentages.

Distributions of Cariogram, "chance of avoiding new caries" by gender, were similar in GI. Furthermore, the “chance to avoid new caries” for all boys in GI was found to be lower. Distribution of “chance of avoiding

Table 2: Distribution of Cariogram Green Sector Percentages in the Groups

	Min (%)	Max (%)	Median (%)	Mean (%)	SD (%)
GI	2	34	9	10.80	7.59
GII	0	89	20	30.96	26
GIII	3	88	35	35.28	21.80

GI-GII: p=0.002, GI-GIII: p<0.001, min: minimum, max: maximum, SD: standard deviation.

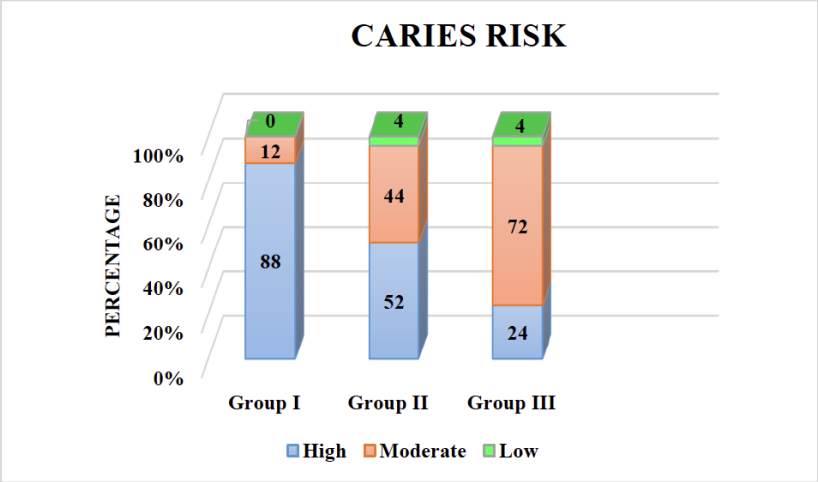

Figure 5: Distribution of Cariogram "chance of avoiding new caries".

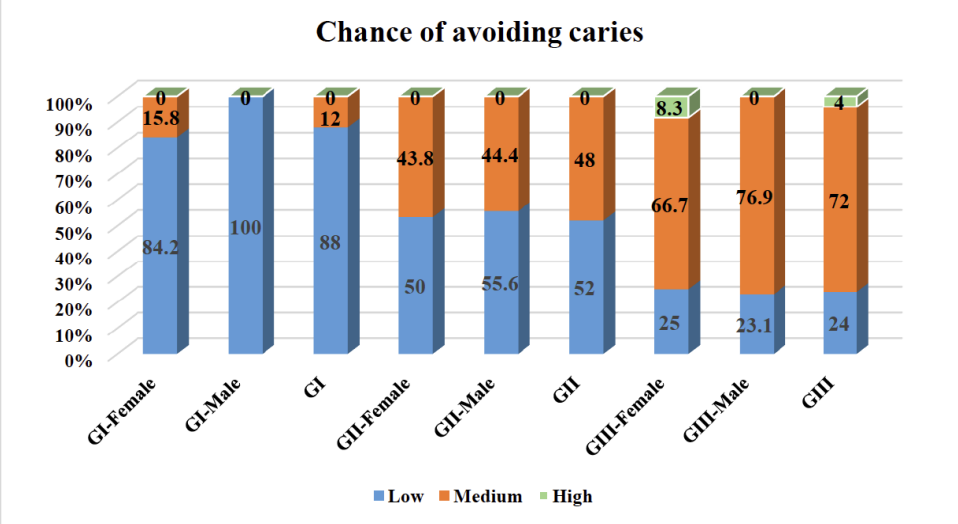

Figure 6: Distribution of "chance of avoiding new caries" by gender in the groups.

new caries" by gender was just assessed as counts and percentages in the other groups because there was insufficient data in the Cariogram software boxes (Figure **6**).

Oral and Dental Health Results and Indices

Inter-group distributions of plaque index variables and also distributions of decayed, missing due to caries, and Filled in the Permanent Tooth/Surfaces (DMFT/DMFS) and decayed and filled primary tooth tooth/surfaces(dft/dfs) indices were determined, and plaque index variables by gender were found to be similar in the groups. In regard to DMFS distributions, there was a significant difference between GI and GIII (p=0.009).

When the presence of enamel hypoplasia was evaluated, hypoplasia was observed in 24% of the

Table 3: Distribution of Numerical Variables in the Groups

Groups		Min	Max	Median	Mean	SD
GI (n:25)	dft	0	10	3	3.28	3.035
	DMFT	0	9	2	2.04	2.28
	dfs	0	28	7	6.80	6.93
	DMFS	0	20	4	4.08	4.95
	Plak index	2.29	2.75	2.61	2.59	0.12
GII (n:25)	dft	0	14	3	3.76	4.12
	DMFT	0	6	3	2.08	2
	dfs	0	27	5	6.84	7.77
	DMFS	0	10	4	3.36	3.53
	Plak index	2.31	2.75	2.61	2.56	0.11
GIII (n:25)	dft	0	8	2	2.56	2.73
	DMFT	0	6	0	0.88	1.62
	dfs	0	14	2	4.12	4.66
	DMFS	0	10	0	1.36	2.58
	Plak index	2.31	2.68	2.58	2.53	0.13

min: minimum, max: maximum, SD: standard deviation.

patients in both GI and GII, whereas %12 hypoplasia was seen in GIII with no D-Vit deficient patients.

DISCUSSION

Vitamin D is very critical for both overall health and dental health. This importance starts from the early stages of dental development and continues through post-odontogenesis affecting the carious lesions. An imbalanced intake of nutrients and energy results in malnutrition due to deficiencies in vitamins, minerals, and protein. The coexistence of vitamin D deficiency and malnutrition necessarily leads to nutritional rickets in children [20].

Vitamin D deficiency in early childhood may have an impact on permanent dentition [20]. Eventually, developed caries may sometimes lead to premature tooth loss and, consequently, to malocclusion and also to chronic periodontal diseases [21,22].

In our study, no significant difference has been found between the severe vitamin D-deficient group (GI) and the group with normal levels of vitamin D (GIII); however, a significant difference has been observed regarding the DMFS distributions (p=0.009). This was believed to be associated with doubled enamel hypoplasia rate in the vitamin D-deficient group compared to the control group. As enamel hypoplasia progresses, bacterial plaque easily accumulates on the teeth surfaces, increasing the rate of caries spread on these surfaces [23-25].

In the present study, as an essential step of Cariogram software modeling, salivary buffering capacity, and flow rate were measured in order to evaluate the caries risk factors. Since the study population consisted of children, stimulated saliva sampling with a shorter duration of collection time was preferred for evaluating the rate of flow. The highest rates of moderate buffering capacity have been observed in all three groups. In light of study data, vitamin D deficiency might be said to have no effect on the salivary buffering rate and flow capacity. In the literature, there is no study evaluating the impact of vitamin D deficiency on saliva; thus, further research assessing the consequences of vitamin D deficiency in the saliva is needed.

In the present study, evaluating the correlation between vitamin D deficiency and caries activity counts, Lactobacillus and S. mutans were found to be similar. A high percentage (60%) of Lactobacillus count was found to be significant in the group with the highest vitamin D-deficiency rates, whereas the highest levels of S. mutans were determined in both control and experimental groups (GI- %88, GII -%72, and GIII-% 72). Elevated counts of lactobacillus are an advanced caries-related parameter [26]. Therefore, the presence

of higher Lactobacillus counts is inevitable in the severe vitamin D-deficient group with elevated DMFS/dmfs scores. No research similar to the present study has been conducted so far assessing the counts of S. mutans and lactobacillus.

In our study, there was a significant difference between the distribution of S. mutans counts by gender in GIII (p=0.03), whereas this distribution was similar in other groups. Likewise, while there was a significant difference between the distribution of lactobacillus counts by gender in GIII (p<0.028), this distribution was similar in other groups.

Cariogram software has been started to be widely used for caries risk assessment in recent years, and interactions between multiple caries-related factors have provided more accurate caries risk prediction [27]. In their 2-year prospective study conducted on 446 children aged 10 to 11, Hänsel Petersson *et al*. [28] evaluated their “change to avoid new caries” with Cariogram. Initially, general health, oral hygiene, and fluoride application data were collected. Saliva analysis was used to calculate S. mutans and lactobacillus counts, and saliva buffering capacities and flow rates were measured. DMFT and DMFS index values were calculated to form bitewing radiographs. Scores were recorded, and caries risk was evaluated. In their study, 8% of the children were determined to have higher risks for dental caries [28]. Wilson *et al*. identified 25% of 84 patients aged 11-12 with a high risk of developing dental caries [29]. And 78% of the children were determined as low-risk patients. Similar to Wilson *et al*.’s [29], the present study also demonstrated a high risk of developing dental caries (24%) in children with normal Vitamin D levels (GIII). Additionally, a high risk of caries was observed in vitamin D deficient (GII) and severe vitamin D deficient (GIII) -groups; 52% and 88%, respectively. Although research for caries risk assessment in healthy children using Cariogram has previously been conducted, our study is the first one evaluating caries risk in vitamin D-deficient children using the Cariogram model. Besides, only a few studies in the literature assess caries risk in patients with systemic diseases using Cariogram [30, 31].

In the present study, percentages of Cariogram green sector were recorded as; between 2 and 34 with a mean of 10.80± 7.59 in GI, between 0 and 89 with a mean of 30.96±26 in GII, and between 3 and 88 with a mean of 35.28± 21.80 in GIII. Regarding Cariogram distributions, significant differences have been observed between GI-GII and GI-GIII (p=0.002, p<0.001, respectively). Obtained data revealed a significant difference between the children with severely low vitamin D levels and others with moderate and normal levels in regard to the percentage of Cariogram green sector.

In other words, as the severity of vitamin D deficiency increased, the percentage of the Cariogram green sector significantly decreased. The Cariogram variable of “avoiding the chance of new caries” was expressed as only counts and percentages due to insufficient data in Cariogram software boxes.

Lower Cariogram percentages of “chance to avoid new caries" observed in GI, GII, and GIII (88%, 52%, and 24%, respectively) implied that as the severity of vitamin D deficiency increased, "chance of avoiding new caries" decreased. Cariogram software used in our study facilitated caries risk assessment in children with lower vitamin D levels

Large-scale population studies are needed for sound generalizability. A major limitation of the present study was that it was carried out with only a small number of patients visiting our clinic. Its second limitation was; since this study was conducted with expensive test kits and an insufficient budget as a part of a research project, only a limited number of samples were involved due to financial issues.

CONCLUSION

Considering that vitamin D deficiency in children increases dental hypoplasia and susceptibility to dental caries, protective measures should be taken; routine vitamin D tests should be performed, and parents should be informed about exposure to natural sunlight, which is the primary source of this vitamin. In case of severe vitamin D deficiency, intake of dietary supplements is essential.

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DECLARATION OF COMPETING INTEREST

The authors have no conflict of interest relevant to this article.

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