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ORIGINAL ARTICLE

Polymorphism in Vitamin D Receptor Gene and Their Relation to *Streptococcus mutans* in Deciduous Teeth Decay among Egyptian Children

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Key words:
Vitamin D gene polymorphism, Streptococcus mutans, decayed, missing and filled teeth index, dental caries.

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ABSTRACT

Background: *Multiple factors play a role in the dental decay occurrence, including microorganisms, diet, oral hygiene, medical conditions, and lack of important nutrients, such as vitamin D.* ***Objectives:*** *correlation between VDR gene and Streptococcus mutans count in children deciduous teeth decay.* ***Methodology:*** *Cross sectional study from January 2018 till January 2019. One hundred and fifty children were involved and divided into two groups; group I: 75 patients with deciduous tooth decay; and group II: 75 children free from dental caries as a control group. Saliva sample for Streptococcus mutans detection and buccal cells sample for vitamin D receptor gene polymorphism (VDR) assay were taken from each patient.* ***Results:*** *S. mutans count was higher in cases than control group and showed statistically significant difference (p=0.0002). Frequency of GG genotype was higher in control (86.7%) than cases group (64.0%) while GT genotype was higher in cases (36.0%) than control group (13.3%). Risk ratio of GT/GG in cases vs control was significant (p=0.001).* ***Conclusions:*** *GT genotype supports the growth of some bacteria in the oral cavity. S. mutans count was higher in GT genotype, so people with GT genotype are more susceptible to develop dental caries by 3.65-fold and suffer from more DMFT. Susceptibility people should increase care of mouth hygiene.*

INTRODUCTION

The presence of one or more decayed, missing, or filled tooth surfaces in any primary tooth in a child 71 months of age or younger is considered as childhood caries. Deciduous teeth erupt after 6 months and tooth decay start in these patients which may be severe in infants and young children and called ECC[1]. Various risk factors inter-relate to increase risk of developing the childhood caries as bacteria, diet type and socioeconomic factors[2]. Mainly *Streptococcus mutans* (*S. mutans*) and Lactobacillus are responsible for this dental caries[3]. Vitamin D is important for increasing the absorption of calcium and phosphate from the food you eat. Vitamin D receptors are found on cells in your immune system and can bind to these receptors and increase the amount of good antimicrobial proteins in your body which help to fight the bacteria that cause dental caries. If the 25-hydroxyvitamin D decreases below 20 ng/ml the Vitamin D deficiency occurs[4]. Vitamin D deficiency causes defective tooth mineralization that may progress to dental caries, delay of tooth eruption, tooth fracture, spontaneous periapical abscesses, widening of the pulp chamber, and dentin dysplasia[5]. The Vitamin D biological function is modulated by VD protein, through interaction with the VDR protein and it has been reported that VDR protein activity is affected by VDR gene polymorphisms[6]. We hypothesized that the VDR polymorphism may be a genetic factor in decidual dental caries and we performed a case-controlled study to determine whether there is an association between polymorphisms in the VDR gene and caries in primary teeth and to detect the correlate of this with the *S. mutans* count.

METHODOLOGY

Patients

One hundred and fifty children (4-6 y) were enrolled in this study. The study population were divided into two groups; group I: 75 patients (36 boys and 39 girls) with deciduous tooth decay (≥ one tooth); and group II: 75 children free from dental caries (41 boys and 34 girls) as a control group who were matched with group I age. The decayed, missing and filled teeth (dmft) index was used to assess tooth decay. 30 Deciduous tooth

decay was diagnosed by visual examination using WHO diagnostic criteria[7].

Samples from patients

Two types of samples were taken from each patient; saliva sample for *S. mutans* detection and buccal cells sample for vitamin D receptor gene polymorphism (VDR) assay. subjects were instructed not to eat or drink anything other than water one hour before collecting the samples. *Salivary samples*: saliva was collected in wide mouth sterile containers and transferred to the laboratory. The collected samples were diluted to ten folds by normal sterile saline and plated on Mitis Salivarius bacitracin Agar (MSB) agar. *Buccal cells samples*: Cheek scraping was done by cotton swab then the tips were swirled for 30-60 seconds in 200 µL of PBS in labeled Eppendorf and then stored at -80 °C. The study was approved by institutional review board of Mansoura faculty of dentistry, Mansoura University.

Isolation and Counting of *Streptococcus mutans*

Mitis Salivarius bacitracin agar is the selective media for *S. mutans*. Tubes including the samples were vortexed for 30 s for content homogenization and each sample was diluted to 10-folds. One hundred microliter of the diluted sample was spread on the surface of MSB agar plates by sterile swab. Cultures were incubated aerobically for 48 h at 37°C under a CO_2 enriched atmosphere. Agar plates were inspected for growth of *S. mutans* colonies. Characterization were done by colonial shape on MSB-agar; Gram-staining film, catalase test, tolerance to 4% sodium chloride, fermentation of mannitol and lactose and esculin hydrolysis test[8]. The *S. mutans*, colony forming units per sample (CFU) were reported.

Extraction of DNA:

It was done from 150 buccal swaps by Thermo Scientific™ GeneJET Genomic DNA Purification Kit, USA, Cat. no #K0781 depending on the manufacturer instructions

Detection of VDR gene polymorphism

The primer pair used according to Morrison et al. protocol was (ACCTGGCCATTGTCTCTCAC) and (CTAACCAGCGGAAGAGGTCA). The DNA banding patterns were visualized under UV light (BioDocAnalyze Digital Compact, Analytik Jena AG, Germany) after staining with ethidium bromide (0.5 mg/mL). BsmI enzyme cuts the PCR product into the following: Homozygous wild genotype (A/A: one band 600 bp); Heterozygous mutant genotype (A/G Three bands 600 bp, 422 bp and 178 bp); Homozygous genotype (G/G genotype represented by one band of 151 bp); and Heterozygous mutant genotype (G/T genotype represented by three bands of 152 bp, 82 bp and 69 bp).[9]

Statistical analysis

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 26.0. Descriptive data was mean ± standard deviation (SD); median, and frequency (Number-percent). Comparison between the different groups, the significance of difference was tested using student's t-test (Unpaired) to compare between parametric data, Mann-Whitney U test was used to compare between non-parametric data and inter-group comparison was by using chi square test (X2-value). Polymorphisms were evaluated by gene counts. Odd's ratio (OR) and confidence interval95% were calculated to detect risk ratio. P value <0.05 was considered statistically significant in all analyses.

RESULTS

Cases and control children were age matched (4.68±0.59 and 4.54±0.63, respectively). The decayed, missing and filled teeth index was 5.00 ± 6.8.

Prevalence of S. mutans in control and cases groups:

S. mutans count was higher in cases than the control and showed statistically significant difference (p=0.0002), as shown in table 1.

Table 1: Prevalence of *S. mutans* in both groups

	Control	Cases	P
S. mutans count	641.33 ± 282.7 (430.0-1500.0)	1217.86 ± 549.8 (500.0-2600.0)	0.0002*

Frequency of different genotypes and alleles in studied groups

Genotyping frequency of GG was higher in control group (86.7%) than cases group (64.0%) while GT was higher in cases group (36.0%) than control group (13.3%). Neither AA nor AG were detected in both groups. Risk ratio of GT/GG in cases vs control was significant (p=0.001). Every increase of GT will increase risk of dental caries by 3.65-fold. Frequency of G allele was higher in control group (93%) than cases group (82%) and T allele was higher in cases group (18.0%) than in control group (7.0%). Risk ratio of T/G in cases vs control was significant (p=0.003). Every increase unit of T will increase risk of teeth caries by 3.07-fold, as shown in table (2) and Figure 1 and 2.

Table 2: Frequency of different genotypes and alleles in studied groups

		Control	Cases	P	OR(CI95%)
Genotype	**GG**	65 (86.7%)	48 (64.0%)	-	1(Reference)
	GT	10 (13.3%)	27(36.0%)	0.001*	3.65(1.61-8.26)
Allele	**G**	140 (93%)	123 (82%)	-	1(Reference)
	T	10 (7%)	27 (18%)	0.003*	3.07(1.43-6.6)

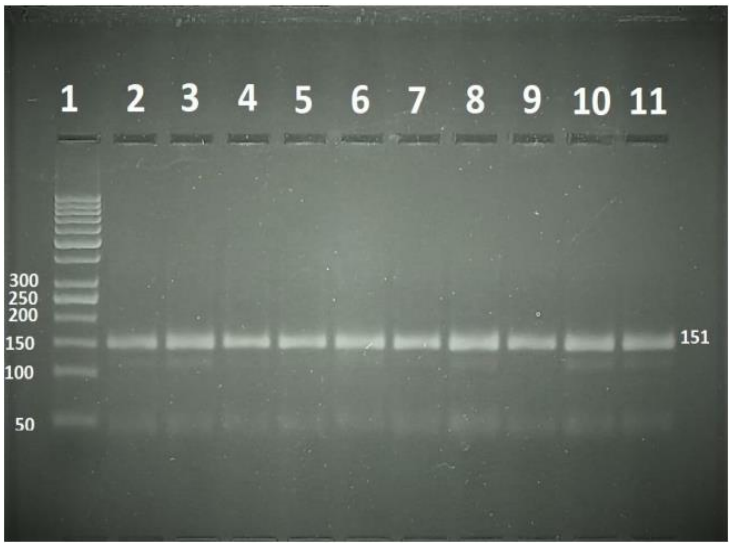

Fig. 1: Agarose gel electrophoresis shows; Lane (1): DNA ladder, and Lane (2-11): G/G genotype of 151 bp band

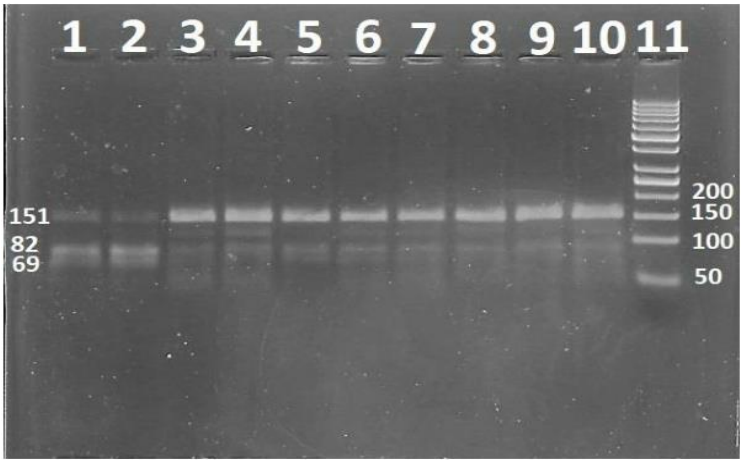

Fig. 2: Agarose gel electrophoresis of BsmI restriction enzyme products; Lane (11): contains a 50 bp DNA ladder, Lanes (1-2): show the (G/T) genotype represented by three bands of 152 bp, 82 bp and 69 bp, Lanes (3- 10): show the (G/G) genotype represented by one band of 151 bp

S. mutans CFU showed statistically significant difference between cases and control regarding GG genotypes (606.7 ±37.4 and control 506.7 ± 101.6, respectively) and GT genotypes (1343.33 ± 615.6 and 729.2 ±237.7, respectively). Also there was significant results between different genotypes in both cases and control group as *S. mutans* CFU was higher in GT than GG, as shown in table (3) and there was significant difference in DMFT between control and case groups in GG & GT genotypes (p=<0.001).

Table 3: Association between *S. mutans* count and different genotypes

	Genotype		P1
	GG	GT	
Cases ***S. mutans count***	606.7 ± 37.4 (500.00-800.00)	1343.33 ± 615.6 (2600.00-600.00)	<0.0001
Control ***S. mutans count***	506.7 ±101.6 (430.00-640.00)	729.2 ±237.7 (600.00-1500.00)	0.001
P2	0.0002	0.001	

*:significance <0.05 Test used: Student's t-test

DISCUSSION

Dental caries is a major oral health problem in most industrialized countries, affecting 60-90% of the population[10].

The incidence of dental caries is influenced by host factors as malnutrition, genetic predisposition, poor health performance, specific eating habits, the presence of organisms affecting tooth decay such as streptococci, fluoride and vitamin D deficiency, excessive sugar consumption and prolonged bottle-feeding, age, gender and place of residence of children[11].

ECC prevention is one of the most pressing and important challenges for dentists and society as a whole. An increased risk of ECC in 3-year-olds was independently associated with the method of breastfeeding within 6 months of age, whereas in 5-year-olds, it was associated primarily with the frequency of food intake before bedtime. ECC has a significant influence on individuals, families, and societies. The disease affects primary and permanent teeth and influences the health and life. ECC is linked with other frequent diseases due to risk factors that shared with other non-communicable diseases[12].

VDR gene controls the vitamin D metabolites function, which plays an important role in tooth formation. The biological function of vitamin D is regulated by the VD protein through interaction with its receptor. It has been reported that protein activity is affected by polymorphisms in the VDR gene[13].

Problem in the VDR gene can lower by 90% the endogenous synthesis of VD or result in VD resistance[14]. It has been reported that there are more than 200 polymorphisms of the receptor genes[15].

Carbohydrates will be metabolized by a certain bacterium, such as *S. mutans* which lead to fermentation and therefore produce copious amount of acid and lower the local pH to a level where the minerals of enamel and dentine dissolve[16].

In the present study, *S. mutans* count was higher in cases than the control group with statistically significant difference and it was higher in GT than GG. This agrees with Sounah and Madfa[17]. Also, Dianawati et al. study showed that among 50 children caries, 94% were colonized by *S. mutans*[18].

The prevalence of caries in primary teeth is usually assessed using the dmft index. The number of carious, and missing primary teeth is calculated such that each child reaches a value known as the average dmft score for that child[19].

In the present study there was no statistically significant difference (p=0.6) in DMFT between GG & amp; GT in cases group. However, there was statistically significant difference in DMFT between control and case groups in GG & GT genotypes (p<0.001). this is similar to Qin, et al.[20].

Natural sequence variants may occur with more than one form, having a frequency greater than 1% in a human population[21]. Hu et al have conducted a relationship between VDR gene polymorphisms and dental caries susceptibility in a Chinese population[22]. The frequency of the TT genotype was observed to be higher in individuals with caries than in those without caries; the "t" allele was considered a caries susceptibility marker. Cogulu et al**.** showed statistically significant difference in the frequency of genotypes (tt)[23]. Aribam et al found no significant difference between the cases and control was observed among the different genotypes and the alleles. However, there was an inclination in the incidence of caries with the genotype 'TT'[24].

In the present study genotyping GG was higher in control group (86.7%) than cases group (64.0%) while GT was higher in cases group (36.0%) than control group (13.3%). Every increase of GT will increase risk of dental caries by 3.65-fold. Every increase unit of T will increase risk of dental caries by 3.07-fold which are near to the results reported by Kong et al. [25].

In contrary to our results, Yu et al conducted a study on 200 dental caries patients and 200 healthy controls aged 12 years were genotyped for *VDR* gene polymorphisms using the PCR-restriction fragment length polymorphism assay. The four polymorphic genes were assessed and showed no statistically significant differences in *VDR* gene polymorphisms between the caries and control groups[26].

CONCLUSIONS

GT genotype support the growth of *S. mutans* in the oral cavity. People with GT genotype are more susceptible to develop dental caries by 3.65-fold.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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