The Role of Vitamin D Receptor Polymorphisms on Dental Caries

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Objective: *To determine the association between the ApaI, FokI, Cdx2 and TaqI polymorphisms of vitamin D receptor (VDR) gene in caries-active (high-moderate) and caries-free children.* ***Study design:*** *A hundred and fifty children (75 males, 75 females, mean age: 10.19 ± 1.61 years) were included in the study. The subjects were divided into three groups as high caries risk group (DMFT, dft>4)(n=55), moderate caries risk group (DMFT, dft=1-4)(n=57) and caries-free group (n=38). From each individual, blood samples were collected and DNA was extracted. The VDR gene was genotyped for the polymorphisms ApaI, FokI, Cdx2 and TaqI using polymerase chain reaction and restriction fragment length polymorphism methods. All data were analyzed by chi-square test, Fisher's exact test and t test.* ***Results:*** *There was statistically significant difference in the frequency of TaqI genotypes (tt) between caries-active and caries-free children (p=0.029). No statistically significant differences were detected between ApaI, FokI, Cdx2 genotypes and dental caries.* ***Conclusion:*** *In the future, VDR gene polymorphisms may be used as a marker for the identification of patients with high caries risk.*

Key words: *Vitamin D receptor, polymorphism, dental caries.*

INTRODUCTION

Dental caries is a major oral health problem in most industrialized countries, affecting 60-90% of the population. Dental caries incidence is affected by host factors that may be related to the structure of dental enamel, immunologic response to cariogenic bacteria, or the composition of saliva.[1-5] Genetic variation of the host factors such as Vitamin D polymorphism may contribute to increased risks for dental caries.[6]

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The Vitamin D endocrine system is central to the control of bone and calcium homeostasis. The active form of the vitamin D is 1,25-dihydroxyvitamin D (calcitriol), the circulating level of which is tightly regulated and acts through a specific receptor to mediate its genomics actions on almost every aspect of calcium homeostasis.[7-9] Genetic alterations of the Vitamin D Receptor (*VDR*) gene could lead to important defects on gene activation, affecting calcium metabolism, cell proliferation, immune function, etc…., which could be explained by changes in the protein sequence.[10-12]

The *VDR* gene locus is found at 12q13.1 region in the human genome. It is about 10 kb and can generate several tissue-specific transcripts[7,8,13] There are more than 200 polymorphisms of the *VDR* gene, including more commonly ApaI, FokI, Cdx2 and TaqI.[14,15]

There has been a lack of reports on the relationship between *VDR* gene polymorphisms and dental caries. Therefore, the aim of this study was to determine the association between the ApaI, FokI, Cdx2 and TaqI polymorphisms of *VDR* gene in caries-active (high-moderate) and caries-free children

MATERIALS AND METHOD

A total of 350 children aged between 6-12 year-old were enrolled in the study. Ethical approval was obtained (06-12/3) and written informed consent was acquired from each parent. Children having any systemic disease were not included to the study.

Tooth-brushing habits (less than once, once, twice), daily dietary sugar exposures (0-2 times , >2 times) were recorded by a structured questionnaire.

Dental examinations of the children were conducted by a pediatric dentist under natural light with the aid of a dental mirror and explorer. Dental plaque scores were recorded according to Silness & Löe index.[16] The mesial, distal, lingual and buccal surfaces of all erupted teeth were scored by one observer on a scale of 0-3. The mean surface score per tooth was determined by dividing the total score of all surfaces by four. Children with the dental plaque score 3 were not included to the study. Among 350 children, 150 children (75 males, 75 females) with similar dental plaque scores, oral hygiene habit and sugar consumption were included to the study to eliminate the other risk factors of dental caries. The DMFT (D: decayed, M: missing, F: filled, T: tooth for permanent teeth) and dft (d: decayed, m: missing, f: filled, t: tooth for deciduous teeth) indices were recorded according to WHO criteria[17] and all children were classified according to the DMFT scores[18]; Group-1: with high caries (dmft/DMFT>4, n=55), Group-2: with moderate caries (dmft/DMFT=1-4, n=57) and Group-3: caries-free (dmft/DMFT=0, n=38).

DNA extraction and genotyping

After obtaining an informed consent, 5 ml venous whole-blood specimens were collected in ethlenediamine tetraacetate (EDTA) tubes and stored at -20°C until processing. DNA (Deoxyribonucleic acid) was extracted from the blood samples by using QIAamp DNA Blood mini kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The *VDR* gene was genotyped for the polymorphisms ApaI, FokI, Cdx2 and TaqI using polymerase chain reaction (PCR). Following PCR reactions, all four polymorphisms (rs7975232-ApaI, rs10735810-FokI, rs11568820-Cdx2 and rs731236-TaqI*)* in the *VDR* gene were genotyped using restriction fragment length polymorphism (RFLP). Amplification was performed in a 20 ml reaction mixture containing genomic DNA (100-150 ng), 200 nM dNTPs (Deoxyribonucleotide) (MBI-Fermentas, USA), 2-8 pmol of each primer and 0.5 U of Taq DNA polymerase (MBI-Fermentas, USA) per tube in a gradient thermal Master cycle (Eppendorf, USA). Amplification reactions (a mechanism leading to multiple copies of a chromosomal region within a chromosome arm) were set up separately for ApaI (AA, Aa, aa), FokI (FF, Ff, ff), Cdx2 (GG, GA, AA) and TaqI (TT, Tt, tt) polymorphic sites of *VDR* gene using specific primers (Table-1). A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis. It is required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA.

Statistical analysis

Allele frequencies, genotype frequencies and carriage rates of the alleles in all groups were compared by using Fisher's exact test. The Hardy-Weinberg equilibrium at individual loci was assessed by c^2test using Statistical Package for the Social Sciences (SPSS) for Windows (version 15.0; SPSS Inc., Chicago, IL, USA) and clinical association was calculated by paired t test. A value $p<0.05$ was considered to be statistically significant.

RESULTS

The mean age of the 150 (75 males and 75 females) children was 10.19 ± 1.61 years. The mean DMFT and dmft scores of all 150 children were; 1.35 ± 0.93 (Group-1: 2.67 ± 1.24, Group-2: 0.26 ± 0.17, Group-3: 0) and 3.67 ± 2.61 (Group-1: 5.76 ± 2.70, Group-2: 0.36 ± 0.30, Group-3: 0), respectively. The mean value of dental plaque score of all children was 1.12 ± 0.10.

Among 350 children, 150 children with similar dental plaque scores, oral hygiene habit and sugar consumption were included to the study to eliminate the other risk factors of dental caries. No significant correlation was detected between DMFT scores and caries risk factors (chi-square test, $p>0.05$). Tooth-brushing habit was regularly twice a day in only 26% (n=39) of these 150 children. Daily sugar consumption was over 2 times *per* day only in 20 % (n=30) of these 150 children. (Table-2).

Table-1. Specific primers and PCR condition

	Specific primers	PCR condition
Apa1	***F:AGCATGGACAGGGAGCAA*** ***R: CCTGTGCCTTCTTCTCTATCC***	15 cycles: 95°C (30 sec), 61.9°C (30 sec), 72°C (30 sec) 20 cycles : 95°C (30 sec), 54.9°C (30 sec), 72°C (30 sec)
Fok1	***F:CCCTGGCACTGACTCTGCTC*** ***R: GGAAACACCTTGCTTCTTCTCC***	30 cycles: 94°C (60 sec), 60°C (60sec), 72°C (60sec)
Cdx2	***F:AGGCAGGAAGGAAGGAAGA*** ***R: CCTTGGTTTGCTTTCATTGC***	30 cycles : 95°C (3 min), 95°C (60 sec), 60°C (60 sec), 72°C (60 sec)
Taq1	***F: CCTGTGCCTTCTTCTCTATCC*** ***R: AGCCTGAGTGCAGCATGA***	30 cycles : 95°C (5 min), 95°C (45 sec), 54°C (45 sec), 72°C (45 sec)

Apa1, Fok1, Cdx2 and Taq1: Studied Vitamin D Receptor polymorphisms

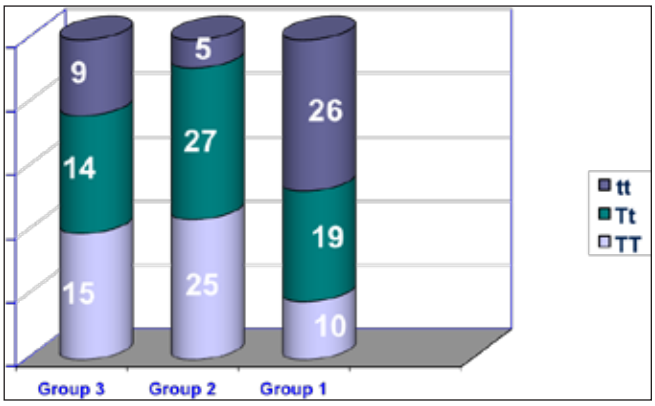
PCR: Polymerase chain reaction

Table-2. The distrubition of caries risk factors according to the groups

	Tooth Brushing (n)			Sugar intake (n)		Dental plaque (n)		
	<1	1	≥2	0-2	>2	0	1	2
Group 1 (DMFT>4)	17	24	14	43	12	8	34	13
Group 2 (DMFT= 1-4)	20	23	14	47	10	7	36	14
Group 3 (DMFT = 0)	10	17	11	30	8	9	20	9

*No significant correlation was found between DMFT scores and caries risk factors ($p>0.05$)

The allele and genotype frequency distribution and carriage rate of *VDR* TaqI polymorphism among patients was given in Figure-1. There was statistically significant difference in the frequency of TaqI genotypes (tt) between caries-active and caries-free children (p=0.029). No statistically significant differences were detected between ApaI, Fok and Cdx2 genotypes and dental caries (p>0.05).



DISCUSSION

Environmental and genetic factors play important roles in the mechanisms involved in the development of dental caries. It is generally accepted that dental plaque scores, oral hygiene habit and sugar consumption are primary etiological factors for dental caries.[1-5] At the beginning of the study, DMFT, dft scores and plaque index were recorded in 350 children and a structured questionnaire was designed. From these children 150 children with similar dental plaque scores, oral hygiene habit and sugar consumption were included to this study to eliminate the other risk factors of dental caries. No significant correlation was detected between DMFT scores and dental caries risk factors.

Studies on genes inducing susceptibility to dental caries have been carried out by various groups in different populations.[19-21] The Vitamin D receptor (*VDR*) gene is a candidate gene for susceptibility to several diseases. *VDR* gene polymorphisms have found to be strongly associated with mineral density. Vitamin D is known to modulate calcium homeostasis and has a role in the regulation of electrolytes and blood pressure. There is now an increasing amount of evidence to show that 1,25 $(OH)_2D_3$, the most active metabolite of Vitamin D, regulates the immune response and possesses anti-inflammatory activity.[22] However, only few data have been published indicating the relationship between *VDR* gene polymorphisms and dental caries.[6] Therefore, we aimed to investigate the incidence of common *VDR* gene polymorphisms (ApaI, FokI, Cdx2 and TaqI) in dental caries.

Vitamin D has special roles in regulating the metabolisms of calcium and phosphorus, and in the immunosystem. The biologic function of vitamin D must be associated with its receptor. Studies demonstrated human *VDR* gene is localized in chromosome 12.[13,23] It is clear that mutations in functionally critical areas of *VDR* gene can have profound effects on mineral metabolism and bone mineral density.[24]

A comprehensive early study showed that vitamin D supplementation significantly reduced the incidence of caries in children. The investigators also noted the substantially lower incidence rate of caries in summer compared to other seasons.[25] It has been shown that the incidence of caries in 12-14 year-old was correlated with the hours of sunshine.[26] A follow-up report showed that 6-8 year-old and 9-11 year-old similarly had caries rates inversely related to ultraviolet exposure.[27]

Caries was strongly associated with bone mineral density and this likely reflected a caries-vitamin D association.[28] Year-round exposure to ultraviolet wavelengths was credited for preventing an increase in caries in school children.[29] Reduced exposure to ultraviolet light was blamed for increase in caries.[30,31] Another study can be planned on the role of *VDR* polymorphim and mineral content of teeth.

A polymorphism is a genetic variant that appears in at least 1% of the population. These changes can ocur in non-coding parts of the gene, so they would not be seen in the protein product. Changes in these regulatory parts of the gene would then affect the degree of expression of the gene, and thus the levels of the protein.[10-12] More than 200 polymorphisms of the *VDR* gene have been reported.[14,15] Among them, most common polymorphisms (FokI, ApaI, Cdx2 and TaqI) have been studied in the present investigation. There was statistically significant difference in the frequency of TaqI genotypes (tt) between caries-active and caries-free children (p=0.029). No statistically significant differences were detected between ApaI, FokI, Cdx2 genotypes and dental caries (p>0.05). Based on the results of this study, in the future *VDR* gene polymorphisms may be used as a marker for the identification of a patient wih high caries risk.

The only data that had been reported in the literature studied the relationship between BsmI and ApaI polymorphisms and dental caries. No significant relationship had been reported in this study.[6] No significant relationship was also found between ApaI polymorphism and dental caries in the present study.

Further studies involving genetic linkage assessment are needed to evaluate the direct effect of these polymorphisms on dental caries; also, the studies should be carried out on a large cohort of population.

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