Vitamin D receptor *TaqI* (rs731236) gene polymorphism in caries patients

A Z Gani¹, P K Zahra¹, N Soedarsono¹, L Yunaini², E I Auerkari¹

¹ Department of Oral Biology, Faculty of Dentistry, University of Indonesia, Jakarta, Indonesia
² Department of Medical Biology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

Corresponding author: ei_auerkari@yahoo.com

**Abstract.** Vitamin D receptor (VDR) is included in the type of protein that serves as the biological function regulator of vitamin D. Tooth formation, especially in enamel and dentin calcification, as well as maintaining the balance of phosphate and calcium ions which is an important factor in protecting teeth requires support from vitamin D. The VDR gene will regulate the activity of VDR proteins. Caries is a multifactorial disease in which genetic factors can affect the host susceptibility to caries. Polymorphism in the VDR gene is suspected to affect the host susceptibility to caries through changes in calcium metabolism. This study aims to discover the VDR gene polymorphism and its association with caries patients in Indonesia. 100 DNA samples from 100 blood samples, including 50 dental caries patients and 50 healthy controls, were analyzed using PCR-RFLP technique. PCR products were digested with the *TaqI* restrictive enzyme, then assessed with statistical analysis using Fisher’s exact test and Continuity correction test. In the caries group, there were no samples with CC genotype, 4 samples with CT genotype, and 46 samples with TT genotype. There were also 4 C alleles and 96 T alleles. Polymorphic genotypes and alleles were found higher in the caries group (100% and 96%) than healthy controls (88% and 84%). These results conclude that the polymorphism of VDR *TaqI* (rs731236) gene was found in patients with dental caries. The distribution of genotypes and allele distributions of VDR *TaqI* (rs731236) gene between caries and healthy controls significantly differs noticeable (p <0.05).

1. **Introduction**

Caries (tooth decay) is a chronic disease caused by an interaction between the tooth surface, biofilm, and sugars from food. The bacteria in biofilm metabolizes sugars and produces acids, which will damage the enamel. Based on FDI data in 2010, the global prevalence of caries was 44% [1]. Global Burden of Disease Study in 2016 estimated as many as 3.58 billion individuals worldwide had oral diseases, in which caries in permanent teeth as the most common condition [2]. In Indonesia, based on data from Riset Kesehatan Dasar (Riskesdas) in 2018, the prevalence of caries was 88.8%, and 56.6% of them were root caries [3].

Many factors can cause caries; therefore, caries is said to be complex and multifactorial. Although its main cause is the interaction between tooth, biofilm, and carbohydrate, the process of caries is not only caused by the interaction, but also involves other risk factors that will affect the host susceptibility to caries [4,5]. The development of caries gets a boost over environmental risk factors, such as low fluoride exposure, inadequate saliva flow, poor oral hygiene, a cariogenic diet, and high amounts of...
Variations in genes (polymorphisms) may cause changes in the proteins they code. Those changes may affect tooth enamel structure, the host’s immune response to cariogenic bacteria, and saliva composition, which will affect the host’s susceptibility to caries [8]. Therefore, genetic polymorphisms may cause some individuals to be more susceptible (risk factor) or resistant (protective factor) to caries than others [5,7–9]. Genetic factors that are associated with caries include genes related to saliva, genes related to taste, immune response genes, and genes that are responsible for enamel formation [7]. One of the genes that have a role in enamel formation is the Vitamin D Receptor (VDR) gene. It regulates the activity of VDR proteins, which are receptors that regulate the biological function of vitamin D. Vitamin D plays an important associate role in tooth formation, particularly in enamel and dentin calcification, in addition to maintaining a balance between phosphate and calcium ions, which are vital factors in protecting teeth. Vitamin D not solely contributes to the management of calcium and phosphate levels. It also has very important functions over the immune system. Vitamin D deficiency may cause changes in the immune system; the immune response to microbial infections in the mouth can also be interfered, such as in periodontitis or untreated caries [9,10]. Variations that occur in the VDR gene may affect the host’s susceptibility to caries through changes in calcium metabolism and host immune response [8].

Single-nucleotide polymorphisms (SNPs) in the VDR gene have been associated with caries in several studies [6,8,10–12]. A case-control study conducted by Hu et al. (2015) in China stated that northwestern China individuals who have the “Tt” genotype possess a higher level of susceptibility to caries [11]. This is similar to the study conducted by Cogulu et al. (2016) in Turkey, which stated that the “tt” genotype in VDR TaqI might increase caries’ risk [8]. In contrast, a study conducted by Holla et al. (2017) in Czech stated that variations that occur in the VDR TaqI gene cannot be used as a marker in identifying caries risk [6].

Many studies have been conducted to understand the association between VDR gene polymorphisms and caries disease in various populations. However, no studies have been conducted in the Indonesian population to date. Therefore, this study was established to determine the distribution of the VDR TaqI (rs731236) gene polymorphism in Indonesian population and its relationship with caries disease.

2. Methods

2.1. Subject selection and DNA isolation

This study was conducted using a cross-sectional study using descriptive study and laboratory analysis. A total of 50 caries patients and 50 healthy individuals were registered. After getting the consent form signed by every participant, 100 stored samples of extracted biological DNA were kept in this present study. All samples were kept at −20°C within the Oral Biology Laboratory in the Faculty of Dentistry, University of Indonesia. The DNA isolation procedure was supported Auerkari et al.’s research [13,14]. This study was under ethical approval from the ethical approval from Faculty of Dentistry’s ethical committee in the University of Indonesia, No.07/Ethical Exempted/FGKUI/VII/2019.

2.2. Genotyping

The genotypes of VDR TaqI (rs731236) gene were analyzed by using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. In this study, the amplification was performed using 20 μL PCR mix consisting of 10 μL MyTaq HS Redmix [Bioline, UK], 0.5 μL forward primer [Integrated DNA Technologies, USA], 0.5 μL reverse primer [Integrated DNA Technologies, USA], 0.2 μL DNA template, and 8.8 μL ddH2O. The primer sequences used to amplify were as follows 5’-AGC AGA GCA GAG TTC CAA GCA GA-3’ and 5’-ATC TTG GCA TAG AGC AGG TGG CT-3’. PCR was conducted with an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, initial elongation at 72°C for 60 seconds, and final elongation at 72°C for 10 minutes. PCR product was electrophoresed in a 1.5% agarose gel.
agarose gel [Genetika Science, Indonesia] at 70V/400mA for 45 minutes and visualized using Gel doc, showing the 472 bp amplification product.

10 μL of the PCR products were digested with 0.2 TaqI restriction enzyme [Thermoscientific, USA], buffer enzyme enzyme [Thermoscientific, USA] (C allele, 380 bp and 92 bp; T allele 472 bp), incubated for 2 hours at 65°C. RFLP, and inactivated for 20 minutes at 80°C using thermoblock. The RFLP product was electrophoresed in 2% agarose gel at 70V/400 mA for 45 minutes and visualized using Gel doc.

2.3. Data analysis
The data obtained were analyzed using the Statistical Program for Social Science (SPSS) v.25 to analyze the differences in the genotype and allele distributions of the VDR TaqI (rs731236) gene between caries patients and healthy individuals. The allele distribution was analyzed using the Continuity correction test, and the genotype distribution was analyzed using Fisher's exact test between caries and the control group, with a p-value < 0.05 was considered significant. Genotype distributions were compared with those expected for samples from populations in Hardy-Weinberg equilibrium using a Chi-square test. The odds ratio was also calculated to determine whether VDR TaqI (rs731236) gene polymorphism might affect the risk level for caries development.

3. Results
The VDR TaqI (rs731236) gene polymorphism was genotyped in a total of 100 samples, containing 50 caries patients and 50 healthy individuals. The genotype frequencies of caries patients and healthy individuals were not in Hardy-Weinberg equilibrium.

Amplified PCR products of VDR TaqI (rs731236) gene that was electrophoresed and visualized using Gel doc showed single bands with a fragment length of 472 bp, as depicted in figure 1. The length of the fragment was measured using 100 bp DNA ladder [Geneaid] as a marker.

PCR products i.e., VDR TaqI (rs731236) gene were then cut using TaqI restriction enzyme to identify each sample’s genotype and allele. The TaqI restriction enzyme will only cut the DNA band on two specific bases, which are thymine and cytosine on 5’...T↓C G A...3’ sequence. Hence, if polymorphism occurs, the DNA band will not be cut off due to the restriction enzyme cannot recognize the sequence. DNA cutting by the TaqI restriction enzyme will generate DNA bands, each of which has a fragment length based on its allele (472 bp for the T allele, 380 bp and 92 bp for the C allele). CC genotype, which is considered wild-type homozygote, will show two DNA bands with fragment lengths of 380 bp dan 92 bp. CT genotype, which is considered a mutant heterozygote, will show three DNA bands with fragment lengths of 472 bp, 380 bp, and 92 bp. TT genotype, which is considered a mutant homozygote, will not be cut; hence it will only show one DNA band with a fragment length of 472 bp. The result of PCR-restriction fragment length polymorphism (RFLP) of VDR TaqI (rs731236) is depicted in Figure 2.

![Figure 1. PCR product visualization](image-url)
VDR TaqI (rs731236) gene polymorphism is identified by a change from cytosine to thymine at intron 9 in the 3’ UTR; therefore, the T allele is considered as a mutant allele (polymorphic allele), and the C allele is considered as a non-polymorphic allele. Consequently, CT and TT genotypes are considered being polymorphic genotypes, while the CC genotype being non-polymorphic genotypes. The estimated number of VDR TaqI (rs731236) gene distribution in the population is established by performing the Hardy-Weinberg test and a value of 0.001 (<0.05) is obtained which indicates that the genotypes and alleles in the population are unlikely to be constant from generation to generation. In the caries group, genotype frequency for CT and TT was 8% and 92% respectively, yet the CC genotype was absent. The allele frequency for C was 4%, and T was 96%. Meanwhile, in the control group, genotype frequency for CC, CT, and TT was 12%, 8%, and 80% respectively. The allele frequency for C was 16%, and T was 84%. CC genotype, which was considered as wild-type homozygote (two bands), was observed only in the control group (12%). TT genotype, which was considered as mutant homozygote (one band), was found to have the highest number (92%) in the caries group. The polymorphic allele had a higher percentage in the caries group (96%) compared to the control group (84%); hence the polymorphic genotype had a higher percentage in the caries group (100%) compared to the control group (88%). Table 1 presents the distribution of VDR TaqI (rs731236) genotype and allele frequencies.

The differences in VDR TaqI (rs731236) gene polymorphism's genotype and allele distribution between caries and control groups were analyzed using Fisher’s exact test and Continuity correction test, respectively. Based on those two statistical test results, it was shown that there were significant differences in the distribution of VDR TaqI (rs731236) gene polymorphism's genotype and allele between caries and control groups (p-value < 0.05).

**Table 1.** The distribution of VDR TaqI (rs731236) genotype and allele frequencies.

| Status   | Control | Caries | p-value |
|----------|---------|--------|---------|
|          | n      | %     | n      | %     |
| Allele   |         |        |         |        |
| Non-polymorphic | C | 16  | 16% | 4 | 4% | 0.01* |
| Polymorphic   | T | 84  | 84% | 96 | 96% |
| Genotype  |         |        |         |        |
| Non-polymorphic | CC | 6   | 12% | 0 | 0% | 0.027* |
| Polymorphic   | CT | 44  | 88% | 50 | 100% |
Table 2 shows the odds ratio calculation to determine whether VDR TaqI (rs731236) gene polymorphism might affect the risk level for caries development. The value obtained was 4.571.

**Table 2. Odds ratio calculation.**

| Exposure | Non-Caries | Caries | OR      | 95% CI      |
|----------|------------|--------|---------|-------------|
| C allele | 16         | 4      | 4.571   | 1.471 – 14.210 |
| T allele | 84         | 96     |         |             |

4. **Discussions**

Caries is a complex disease and is caused by many factors (multifactorial). The risk of developing caries may be influenced by genetic factors, in addition to environmental factors such as oral hygiene and diet [6–8,15]. One of the genetic factors that may influence the host’s susceptibility of developing caries is Vitamin D Receptor (VDR) gene [8–10]. Polymorphisms occurring in the VDR gene may alter the amount of VDR protein produced. VDR protein itself plays a role in mediating the action mechanism of vitamin D [10,16]. Vitamin D (active form: calcitriol [1,25(OH)2D3]) plays an important role in the formation and protection of teeth. It regulates calcium metabolism, promotes the deposition of calcium to enamel, and maintains phosphate and calcium ions' stability. Low vitamin D levels in the serum have been shown to be associated with caries [9,10,17].

Polymorphisms that occur in the TaqI VDR (rs731236) gene on the 3' UTR intron 9 section were characterized by the alteration from cytosine base to thymine. Variations that occur this region may affect the mRNA stability, resulting the effect on translated protein amount [10].

In the Indonesian population, the TT genotype and the T allele were found to dominate in both population groups, both in the case and control groups. This result are consistent with research conducted in Korea and in Poland. In a study conducted by Seo et al. (2010) in Korea, the TT genotype and the T allele were also found to dominate in both population groups, while study established by Laczmanska et al. (2014) in Poland found that only T allele which dominate in both population groups with CT genotype dominated the control groups and TT genotype dominated the case groups. [18,19]. In the other hand, there are differences in results between research conducted in Indonesia and in China and Tunisia. Research conducted in China by Yu et al. (2017) and in Tunisia by Maalmi et al. (2013) reported that the CC genotype and the C allele predominate in both population groups, both in the case and control groups [9,20]. In addition, CT genotype was also found to predominate in both population groups of Tunisians [20]. The variation in the results of the distribution of genotypes and alleles in these studies may be influenced by the differences in race and ethnicity in each study population.

Continuity correction test was performed to analyze the allele distribution, and Fisher’s exact test was performed to analyze the genotype distribution in caries and control groups. According to the statistical tests performed, p-value <0.05 were obtained (0.01 and 0.027, respectively). Hence, the proposed hypothesis is acceptable: there are statistically significant differences in VDR TaqI (rs731236) gene polymorphism’s genotype and allele distribution between caries patients and healthy individuals in Indonesia. Nonetheless, according to the Hardy-Weinberg analysis, a p-value of 0.001 (<0.05) was obtained. Therefore, the population’s genotypes and alleles are unlikely to be constant from generation to generation. A similar study that was conducted by Yu et al. (2017) in China found that there were no statistically significant differences in VDR TaqI (rs731236) gene polymorphism’s genotype and allele distribution between both groups, with the p-value obtained were <0.05 (0.089 and 0.106, respectively). This inconsistency may be due to the racial and ethnic differences of the samples used.

The calculation of odds ratio was also performed, and a value of 4.5714 with 95% CI (1.471-14.210). Thus, it can be assumed that individuals in population who have the T allele in the VDR TaqI (rs731236) gene are 4.6 times more likely to develop caries.
5. Conclusions

Based on the obtained research results, we successfully investigated that a VDR TaqI (rs731236) gene polymorphism that presents in caries patients (with T allele as a polymorphic allele) occurs to be the increased risk factor of caries.

This research is a preliminary study to determines the relationship between the VDR TaqI (rs731236) gene polymorphism in caries patients in Indonesia. Therefore, we suggest further research to be conducted, with data such as gender, age, and ethnicity, as well as the other risk factors and protective factors associated with caries be considered.

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