Single nucleotide polymorphism in vitamin D receptor gene and dental caries

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ABSTRACT

Ubiquitous nature of dental caries in all the populations globally, varies in between and in populations due to its complex nature. The fact that it cannot be prevented and its onset may be at any point of time is not constant throughout the course of life. Among the various factors that affects tooth formation and maintenance vitamin D also plays a major role by actively participating in the formation, mineralization and protection of tooth surface through antimicrobial peptide production through various immune responses. The antimicrobial responses are conducted through antimicrobial peptides. In the present study we intend to evaluate the vitamin D receptor gene polymorphism (rs731236, rs7975232, rs 1544410) and its association with dental caries prevalence. After obtaining approval from institutional ethical committee the study was conducted among 376 adults reporting to the outpatient department of Endodontics. They were further divided into caries free and caries active group based on their caries experience. Further 5ml of unstimulated salivary samples were collected and analyzed for salivary Vitamin D and LL-37 levels. Salivary DNA isolation was done and PCR RFLP was conducted for VDR gene SNP's Taq1,Apa1 and Bsm1.
The estimation of vitamin D levels revealed that individuals in caries free group(N=138) showed significantly higher vitamin d levels in comparison to caries active group (p=0.000, student 't' test) with a mean value of 29.44 ng/ µl in caries free and 20.02 ng/ µl in caries active group. The assay showed no significant variation in salivary cathelicidins (LL-37) levels in both the groups even the mean value in caries free is higher i.e., 7.07 ng/ µl. There is a significant association between dental caries and VDR Taq1 polymorphism(p=0.000) and VDR Apa1 polymorphism (p=0.000), there is no significant association between VDR Bsm1 polymorphism and dental caries(p=0.973).

PROTOCOL CITATION

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MATERIALS TEXT

Questionnaire: Baseline data was obtained which included detailed demographic data(age,sex,height,weight) and dietary habits(Non veg/veg) and oral hygiene practice details.
Dental examination: Teeth that were decayed, missing, filled due to caries were assessed and recorded according to oral health survey,2013 criteria[Annexure I]. Two indices were monitored DMFT and PUFA[22,23].PUFA[DSV1]index measures the presence of oral condition resulting from untreated caries ,This indexgives a gist of extent of severity of dental caries than merely focusing on presence or absence of caries.
[DSV1]Expand first tme

Saliva Sample Collection: Unstimulated saliva samples will be collected by Navazesh Protocol,1993 [24]. Subjects will be asked to abstain from smoking brushing of teeth, use of mouthwash, eat/drink for 2 hour prior to the sample collection
Salivary DNA isolation: Salivary DNA was isolated using commercially available kits (NORWGEN SALIVARY DNA ISOLATION KIT[DSV1]) as per manufacturer's instructions. The DNA concentration per sample was analysed using a bio-spectrophotometer and stored at -200C until further analysis.
[DSV1]Put the manufacturer name, serial no, and country of origin

SNP selection and genotyping: SNP was selected based on previous publications and obtained from database http://www.ncbi.nlm.nih.gov. DNA was amplified by polymerase chain reaction (PCR) and examined (by specific restriction enzymes) using the restriction fragment length polymorphism (RFLP) technique. The primer pair for SNP's is mentioned in the given table (Table 1) The 25 µl PCR reaction mixture consisted of10 mM trisHCl, 200 µM dNTPs, 20 pmol from the primer 1.5 mM MgCl2, 0.5 u taq polymerase (fenzyme), and using 50–100 ng of DNA as template.

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1 5ml of unstimulated salivary samples were collected and analyzed for salivary Vitamin D and LL-37 levels. Salivary DNA isolation was done and PCR RFLP was conducted for VDR gene SNP's Taq1,Apa1 and Bsm1

2 Collected data was summarized by frequency, percentage, mean and standard deviation. Comparison was done using chi square test, ANOVA and t test. Logistic regression analysis was performed to obtain the odds ratio of genotype with cases. ROC analysis was performed to obtain the optimum cut off vitamin D level with appropriate sensitivity and specify.

Methodology

3 The estimation of vitamin D levels revealed that individuals in caries free group(N=138) showed significantly higher vitamin d levels in comparison to caries active group (p=0.000, student 't' test) with a mean value of 29.44 ng/ µl in caries free and 20.02 ng/ µl in caries active group. The assay showed no significant variation in salivary cathelicidins (LL-37) levels in both the groups even the mean value in caries free is higher i.e., 7.07 ng/

µl. There is a significant association between dental caries and VDR Taq1 polymorphism(p=0.000) and VDR Apa1 polymorphism (p=0.000), there is no significant association between VDR Bsm1 polymorphism and dental caries(p=0.973). In VDR gene SNP's Taq1 , CC is the protective factor, and allele 'C' is associated with control and allele 'T' is associated with cases. In SNP Apa1 , Allele 'A' is associated with controls and therefore is the protective factor and allele 'C' is associated with cases. The multiple comparisons show a significant association between VDR Taq1 polymorphism and salivary vitamin d levels in caries active group (p=0.05), there was no significant association between salivary vitamin D and VDR Apa1, Bsm1 polymorphisms.