Association of Fok 1 (Rs10735810) Gene Polymorphism with Dental Caries in Pakistani Adolescents

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ABSTRACT

***Objective*:** To determine the association of single nucleotide polymorphism of vitamin D receptor gene Fok 1 (rs10735810) with dental caries in Pakistani adolescents.

***Study Design*:** Case-control study.

***Place and Duration of Study*:** Biochemistry Department, Islamic International Medical College, Rawalpindi in cooperation with Pathology Department, Railway General Hospital, Rawalpindi Pakistan, from Sep 2019 to Aug 2020.

***Methodology*:** A total of 304 participants were included in the study. There were 152 dental caries cases and 152 normal age-matched controls. After taking written informed consent, blood samples were collected from each participant. DNA extraction was done by Chelex Method. PCR was carried out to determine the respective allelic frequencies of Fok 1 (rs10735810) genotype using specific primers.

***Results*:** Out of 304 study participants, there were 26 males (17.1%) and 126 females (82.9%) in case-group; and 37 males (24.3%) and 115 females (75.7%) in control-group. No significant association of Fok 1 (rs10735810) genotype with DMFT score was found in dental caries case-group. Regarding genotypic and allelic frequencies, there was no significant association of Fok 1 (rs10735810) genotype with dental caries case-group.

Conclusion: Single nucleotide polymorphism of vitamin D receptor gene Fok 1 (rs10735810) is not associated with dental caries in Pakistani adolescents.

Keywords: Decayed missing filled teeth score (DMFT Score), Dental caries, PCR, Receptor gene, Single nucleotide polymorphism, Vitamin D.

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INTRODUCTION

Dental Caries (tooth decay) is an infectious disease caused by oral bacteria, mostly Streptococcus mutants and less commonly Lactobacilli.[1] It is the third most prevalent oral disease globally.[2,3] DMFT (decayed missing filled teeth) index determines dental caries prevalence. It is performed using a dental mirror and probe to assess the number of decayed, missing (due to caries) and restored teeth.[4] Low risk of dental caries has been associated with vitamin D supplementation.[5] Single nucleotide polymorphism (SNP) of vitamin D receptor genes are associated with many diseases, including dental caries.[6] SNP is a single nucleotide substitution in a genome that helps to determine the association between gene variants and common diseases.[7] There are two translation initiation sites (ATG) in vitamin D receptor gene.[8] Fok 1 (rs10735810) is a start codon polymorphism that changes nucleotide sequence (ACG). Hence one site elimination gives rise to a three amino acids shorter vitamin D receptor protein, i.e., 424 amino acids instead of 427.[9] Fok 1 (rs10735810) gene polymorphism has two different alleles, F allele (C allele) and f allele (T allele) based on absence or presence of restriction site.[8] The shorter protein resulting from the FF (CC) genotype is more active than the ff (TT) genotype 10. Fok 1 (rs10735810) gene polymorphism is associated with various dental diseases.

In Pakistan, steps can be taken to prevent dental caries by identifying associated risk factors, including genetic testing. The objective of this study was to determine the association between SNP of vitamin D receptor gene, Fok 1 (rs10735810) with DMFT score in dental caries cases of Pakistani adolescents.

METHODOLOGY

It was a case-control study carried out at the Biochemistry Department, Islamic International Medical College, Rawalpindi in cooperation with the Pathology Department, Railway General Hospital, Rawalpindi after approval from the Ethical Review Committee of Islamic International Medical College (Ref no. Riphah/IIMC/IRC/19/0350) (Appl no. Riphah/IRC/19/0372). The duration of the study was one year, from September 2019 to August 2020.

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The sample size was calculated according to the formula; $n=Z^2\ p(1-p)/e^2$, where Z was taken as 1.96 and prevalence as 60%.[10] The calculated sample size was 300.

Inclusion Criteria: Subjects of either gender, up to 12 years of age with dental caries were included in the study as the case-group. The study also included normal age-matched controls.

Exclusion Criteria: Non-consenting subjects were excluded.

A total of 304 samples were collected through non-probability consecutive sampling technique. There were 152 cases of dental caries in the first group and 152 subjects in the normal age-matched controls. Blood samples were collected and transported to the laboratory in EDTA containing vacutainers and refrigerated at 4-8°C. DNA extraction was carried out by Chelex Method. Storage of extracted DNA was performed at-70°C temperature. Fok 1 (rs10735810) gene was genotyped by polymerase chain reaction and primers were used to amplify the gene of interest (Table-I).

Table-I: Primer sequence of Fok 1 (rs10735810) gene.

Primer Name	Primer Sequence 5′ to 3′
FokI/F/ G	TGGCCGCCATTGCCTCCG
FokI/f/ C	TGGCCGCCATTGCCTCCA
FokI/C	AGCTGGCCCTGGCACTGA
DRB1F	TGCCAAGTGGAGCACCCAA
DRB1R	GCATCTTGCTCTGTGCAGAT

PCR was performed in separate tubes. The final total volume was 25µl for each PCR reaction. This 25µl volume comprised of 8.5µl PCR water, 12.5µl Thermoscientific master mix, 1µl primer mix and 3µl of DNA from a sample that had to be genotyped.

At the beginning of PCR amplification, an initial denaturation was performed at 95°C for 5 minutes. This was followed by 30 amplification cycles. Each cycle consisted of denaturation at 95°C for 30 sec, annealing at 61°C for 30 seconds and then extension at 72°C for 30 secends. After the completion of 30 cycles, there was a final extension that was carried out at 72°C for 7 minutes and the amplification was terminated to hold at 4°C. The PCR products were subjected to electrophoresis on 2% agarose gel pre-mixed with Ethidium bromide 1% solution in 1x TBE buffer solution. The duration of electrophoresis was 40 minutes with at 700 amp current and 100V voltage setting. Amplified bands were visualized by UV320 trans-illumination under UV camera in Gene Box by Gene Sys. The amplified bands were compared to Gene Ruler 50 bp (base pair). DNA ladder was used as a reference to determine the size of amplified bands in base pairs (Figure).

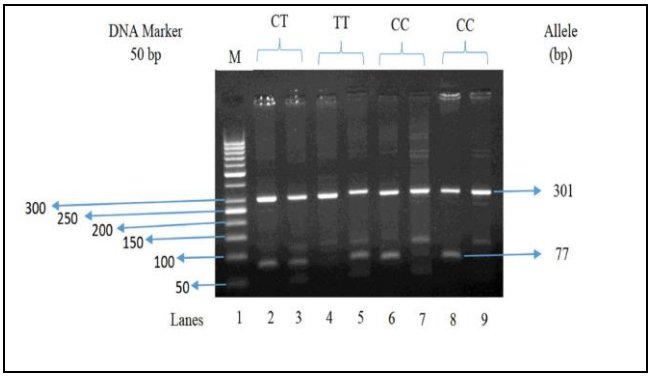

Figure: Electrophoretogram on 2 % agarose gel showing amplified PCR products of Fok 1 (rs10735810) gene polymorphism with DNA marker of 50 bp. Lane 1 represents DNA marker of 50 bp. Lanes 2 & 3 represent CT genotype in cases. Lanes 4 & 5 represent TT genotype in controls. Lanes 6 & 7 represent CC genotype in cases, and lanes 8 & 9 represent CC genotype in controls.

Statistical Package for Social Sciences (SPSS) version 21 was used for the data analysis. Frequencies and percentages were determined for qualitative variables. The Chi-square test was performed to determine possible associations between the Fok1 (rs10735810) gene and dental caries by computing odds ratio (OR) and 95% confidence intervals (CIs). The *p*-value of ≤0.05 indicated statistically significant difference.

RESULTS

A total of 304 participants were included in our study. Out of these, 152 were the cases of dental caries and 152 were normal age-matched controls. There were 26 males (17.1%) and 126 females (82.9%) in case-group and 37 males (24.3%) and 115 females (75.7%) in control-group. There were 14 subjects (9.2%) in 6-9 years age group, 37 subjects (24.3%) in 10-11 years age group and 101 subjects (66.4%) were 12 years old in both the cases and controls. Dental caries was graded using DMFT score to count the number of decayed, missing and filled teeth in each participant. No association was found between Fok 1 (rs10735810) genotype with DMFT score in dental caries cases (p=0.400) as shown in the Table-II.

In terms of genotypic frequencies, no significant association was found between Fok 1 genotype (rs10735810) and dental caries [OR=1.308 (95% CI: 0.817–2.094), p=0.261] as shown in the Table-III.

Regarding allelic frequencies, a lower frequency of allele C was found in the cases (n=238) compared to controls (n=249). On the other hand, a higher frequency of allele T was found in the cases (n=66) than in

Table-II: Association of fok 1 (rs10735810) genotype with decayed missing filled teeth score (dmft score) in dental caries cases.

Genotype in DMFT Score n=152	Decayed Missing Filled Teeth Score (DMFT score)							*p*-value
	1 DMFT 93 (61.2%)	2 DMFT 38 (25%)	3 DMFT 10 (6.6%)	4 DMFT 5 (3.3%)	5 DMFT 3 (2%)	6 DMFT 2 (1.3%)	7 DMFT 1 (0.7%)	
CC	46 (49.5%)	25 (65.8%)	8 (80%)	5 (100%)	2 (66.7%)	2 (100%)	1 (100%)	0.400
CT	44 (47.3%)	13 (34.2%)	2 (20%)	-	1 (33.3%)	-	-	
TT	3 (3.2%)	-	-	-	-	-	-	

controls (n=55). However, we did not find any significant association of allele T with dental caries [OR= 1.255 (95% CI: 0.842–1.872), *p*=0.263] as shown in the Table-III.

Table-III: Fok 1 (rs10735810) genotype of dental caries cases and controls.

	Cases n=152	Controls n=152	OR (95% CI)	*p*-value
Genotype				
CC	89 (58.6%)	99 (65.1%)	Ref	
CT	60 (39.5%)	51 (33.6%)	1.308 (0.817 - 2.094)	0.261
TT	3 (2%)	2 (1.3%)	1.668 (0.272 - 10. 215)	-
Dominant Model				
CC	89 (58.6%)	99 (65.1%)	Ref	
TT + CT	63 (41.5%)	53 (34.9%)	1.322(0.831 - 2.103)	0.238
Recessive Model				
CC + CT	149 (98.1%)	150 (98.7%)	Ref	
TT	3 (2%)	2 (1.3%)	1.510 (0.249 - 9.168)	-
Alleles				
C	238 (78.3%)	249 (81.9%)	Ref	
T	66 (21.7%)	55 (18.1%)	1.255 (0.842 - 1.872)	0.263

DISCUSSION

Dental Caries is associated with many environmental and genetic factors.[11] Frequent consumption of diets containing high sugars, especially candies and chocolates, promotes dental caries.[12] Other factors like poor oral hygiene, insufficient fluoride intake and prolonged bottle-feeding also contribute towards the dental caries.[13] If precautionary measures are taken earlier, dental caries can be avoided at an early stage.

Vitamin D is involved in the development of enamel.[14] Vitamin D deficiency during development leads to defective enamel formation.[14] Vitamin D deficiency alters the immune response, predisposing to bacterial infections.[15] Supplementation with vitamin D has been found to reduce the dental caries.[16] Based on these findings, it was hypothesized that vitamin D receptor genes might be associated with dental caries. Fok 1 (rs10735810) gene polymorphism has been used in many studies as a genetic marker to determine its association with calcium metabolism-related diseases.[9,10,16] Therefore, we conducted a study to determine whether vitamin D receptor gene polymorphism Fok 1 (rs10735810) was associated with dental caries or not.

No significant association was noted in DMFT score with dental caries. Likewise, no association was found between Fok 1 (rs10735810) genotype and dental caries. This result was consistent with the studies conducted in China, Turkey, and Brazil.[5,17,18] However, it was not in accordance with the results of Yu *et al*, on 400 Chinese adolescents where a significant association of Fok 1 (rs10735810) genotype with dental caries was found.[6]

Out of three genotypes, a lower frequency of CC genotype was found in the cases as compared to controls. This finding was in accordance with the study conducted on 353 Brazilian children aged 8-11 years.[18] However, the study conducted by Yu *et al*, on 12 years old Chinese adolescents showed a higher frequency of CC genotype in cases as compared to controls.[6]

A higher frequency of allele T was found in the cases as compared to controls in our study. Therefore, it showed that allele T might be a risk factor for dental caries. However, no significant association between allele T and dental caries was found. This finding was in agreement with the study conducted on 380 Chinese children,[5] where the frequency of allele T was found to be higher in cases and no significant association between allele T and dental caries was found .

LIMITATIONS OF STUDY

Environmental risk factors such as tooth brushing duration, tooth brushing frequency, frequency of sugar intake, use of fluoridated toothpaste, use of dental floss, etc. were not considered in the study. Secondly, other single nucleotide polymorphisms of vitamin D receptor genes like Bsm 1, Taq 1 and Apa 1 can also be evaluated to see the combined effects of four genotypes.

CONCLUSION

Single nucleotide polymorphism of vitamin D receptor gene Fok 1 (rs10735810) is not associated with dental caries in Pakistani adolescents.

Conflict of Interest: None.

Authors' Contribution

BK: Data collection drafting of manuscript & statistical analysis, AR: Critical review of content & study planning, MA: Lab support & PCR optimization, SA: Sample collection, MFJ: Patient counseling & sample collection.

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