Original Article

Single Nucleotide Polymorphisms of Salivary Human Beta Defensins 1 in Relation to Dental Caries

Elaf Abdulkareem Alidan, Ahlam Taha Mohammed

Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad, Baghdad, Iraq

Abstract

Background: The role of genetics in determining an individual's susceptibility to caries is significant, with the human Beta-Defensin 1 gene (*DEFB1*), responsible for encoding the human β-defensin 1 (hBD1) antimicrobial peptide, being a potential candidate for studying genetic susceptibility to caries due to its constitutive expression. Genetic variations in *DEFB1* have been associated with impaired production of hBD1, increasing the vulnerability to oral pathogen infections and potentially leading to dental caries. **Objectives:** As hBD1 localizes in the oral cavity, the objective of the study is to investigative level of salivary Beta defensins 1 and genetic variation that could contribute to caries susceptibility among adolescents in Baghdad city. **Materials and Methods:** A case–control study was conducted among 78 adolescents (39 very low caries and 39 high caries) aged 13–15 years old in Baghdad city. Three single nucleotide polymorphisms (SNPs) were identified from unstimulated saliva samples, namely rs1799946, rs5743418, and rs11362. Saliva collection was done, and then dental caries was evaluated with the D1-4MFS/D1-4MFT (decayed, missing, filled surface/teeth) index. **Results:** There were high concentrations of salivary B-Defensin 1 in control group (very low caries group) compared to study group (high caries group) with significant difference ($P = 0.038$). It also showed that no significant difference was found between the study and the control groups in genotype distribution except for SNP (rs5743418) there was significant difference in heterozygous genotype (CT) and no significant deviation from Hardy-Weinberg equilibrium in genotype distribution of both groups. *DEFB1* SNPs were significantly associated with the caries experience: the strongest association emerged from rs5743418 SNP ($P = 0.006$). **Conclusions:** The rs5743418 SNPs of DEFB1 gene and its level in saliva had a relation with caries experience suggesting that these could be considered as potential marker for assessing the risk for developing caries.

Keywords: Allele, beta defensins 1, dental caries, genetics, health risk, single nucleotide polymorphisms

Introduction

Cariology, the field of dental research focused on dental caries, is known for its extensive complexity. Dental caries, the most widespread infectious oral disease in humans, is a multifactorial disease, related to many factors and influenced by various factors such as lifestyle, obesity, socioeconomic, pregnancy, genetic factors and characteristics of the oral environment.[1-4] In spite the development of techniques to prevent and treat it, dental caries still concerns major problem which can affect the quality of life.[5,6] The development of caries requires a susceptible host to be exposed to cariogenic microorganisms and carbohydrates for prolonged periods, along with the impact of host factors such as the flow rate and buffering capacity of saliva, tooth positioning, enamel surface characteristics, and occlusal fissures depth.[3,7] *In vitro* studies had revealed that different salivary proteins, such as lysozymes, interleukins, mucins, and lactotransferrin, can play various roles in promoting cell aggregation, inhibition, and/or bacterial adherence.[8,9] Additionally, certain proteins, such as human beta defensin 1 (hBD1), exhibit direct antibacterial effects.[10] These findings highlight the diagnostic and interventional potential of salivary proteins and peptides in various clinical scenarios,[11] which may pave the way for the

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_467_23

Address for correspondence: Dr. Elaf Abdulkareem Alidan, Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad, Baghdad, Iraq. E-mail: elaf.ali1901@codental.uobaghdad.edu.iq

Submission: 24-Apr-2023 **Accepted:** 29-Jun-2023 **Published:** 23-Jan-2026

How to cite this article: Alidan EA, Mohammed AT. Single nucleotide polymorphisms of salivary human beta defensins 1 in relation to dental caries. Med J Babylon 2025;22:1062-9.

development of prevention programs or personalized treatment approaches.[12]

Numerous studies have revealed that despite controlling and standardizing environmental factors, there still exists variability in susceptibility to dental caries among individuals. This suggests that certain environmental factors may have a higher cariogenic effect on some individuals compared to others.[13-15] This variability in susceptibility could potentially be explained by genetic differences among individuals, as genetic influences can modify the expression of the disease in each individual.[16]

In Iraq, studies established to link the genetic role in periodontal disease and only explored the level of hBD1 in saliva[17,18] but no previous studies in Iraq have linked the genes of antimicrobial elements, such as hBD1 in saliva with dental caries. Therefore, the current study aimed to identify and screen susceptible patients, and to understand the contribution of genes in the pathogenesis of caries. As hBD1 is localized in the oral cavity, we conducted tests to investigate whether variations in *DEFB1* gene are associated with caries.

Materials and Methods

This comparative cross- sectional study was done in a specialist dental health center in Al-Khadymia/Baghdad city for 8 months period (from August 2021 until April 2022); subjects' sample was selected from patients who attended the center. Informed consent was taken from the patients' guardian before the examination. The unstimulated saliva collection and oral examination were performed from unrelated 78 individuals (39 very low caries and 39 high caries) and analyzed for three single-nucleotide polymorphisms (SNP) in DEFB1 in DNA samples.

Inclusion criteria

Patients within the age range of 13–15 years old regardless of gender, and they should be Iraqi Arab population with no history of any systemic diseases or medications in the preceding 3 weeks. They should have high caries scores with DMFT more than 9 and low caries scores persons with DMFT less than 2.[19]

Exclusion criteria

Patients with any systemic disease, cleft, congenital anomalies, generalized dental problems or who wore a fixed orthodontic appliance. Patient received fluoride supplements or had fissure sealant. Subjects with DMFT more than two but less than nine were not included in the current study.

At first, unstimulated saliva was collected from each participant and then oral examinations were done to ensure that examination did not make any stimulation. The unstimulated saliva was collected for 5 min. Participants were asked to spit the saliva through a funnel into a scaled sterilized tube every one minute. After saliva collection, the samples divided in two parts; one of them used to detect hBD1 concentration was being centrifuged at 4000 revolution per minute (rpm) for 5 min in order to separate the mucins. The clear supernatant was separated by micropipette and was stored at a temperature of about –80°C according to the manufacturer instructions (MyBioSource, San Diego, United States). The other part used to extract DNA was being centrifuged at 12,000 rpm for 3 min.

Clinical examination was conducted using disposable mouth mirror and dental explorer after drying of the field of examination with air triple. Dental caries was evaluated with the DMFT/DMFS (decayed, missing, filled teeth) index, for each patient as recommended by World Health Organization guidelines,[20] for the severity of dental caries according to criteria of WHO (1976).[21]

DNA extraction and quantitation

Genomic DNA was isolated from saliva sample according to the protocol ReliaPrep™ Saliva gDNA Miniprep System, Promega. Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the quality of samples for downstream applications.

Primer preparation, optimization and PCR amplification

The primers were supplied by Macrogen Company in a lyophilized form. The DNA template was amplified with the same primer pair (Forward) (Reverse). After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

Standard sequencing

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation (Korea). The results were received by email and then analyzed using geneious software.

ELISA assay for measuring β-defensin 1 levels

The saliva obtained from participants was analyzed for hBD1 using commercially available ELISA kits following the manufactures' instructions (MyBioSource, San Diego, United States).

Statistics

Computerized software statistical package for social science (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp) was used. The variation of frequencies between groups was analyzed using Chi-square test. Hardy-Weinberg equilibrium (HWE) was used to calculate the expected common homozygotes, expected heterozygotes, and expected rare homozygotes. Chi-square test was used to find out genotype deviation from HWE, and to compare the distributions of genotypes and allele frequencies in the disease and control groups. The relative risk (RR)

is the real measure of association between exposure to a certain factor and having the disease or outcome. The risk associated with individual genotypes or alleles was calculated as the odds ratio (OR) with 95% confidence intervals (95% CI), which indicate how many times more frequently a disease develops in individuals carrying the allele or genotype than in individuals lacking it.

Spearman's coefficient correlation (r) was used to find correlations between hBD1 and dental caries.

Ethical approval

The study was conducted in accordance with the ethical principles that had their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee of Baghdad University/College of Dentistry according to the document number 366 (including the number and the date in August 1, 2021) to get this approval.

Results

Genetic analysis of hBD1 SNPs

Agarose gel electrophoresis of PCR products and sequencing

The genetic analysis was done for hBD1 SNPs gene polymorphisms and seven polymorphisms were detected according to primer design which was higher at 852bp; the result of PCR is shown in Figure 1.

Genotype and Allele frequency analysis for DEFB1 gene

The genotype and allele frequency comparisons calculated for hBD1 SNPs are illustrated in Tables 1 and 2. These showed that no significant difference between the study and control groups except for SNP (rs5743418) there was significant difference in genotype CT. These differences in genotype between two groups are indicating possible correlation of this marker with caries in children. There was no significant deviation from HWE in genotype distribution of both groups.

The frequency of the major alleles was higher in low caries group than high caries in all SNPs except for rs11362. The significant difference was found between high and low caries groups in rs5743418 and in low caries group the SNP rs1799946 revealed a significant deviation from HWE between G and A alleles ($P = 0.015$) and that mean the G allele is a protective factor for developing caries. In high caries group, rs11362 revealed a significant deviation from HWE ($P = 0.005$) and that mean the G allele is a risk factor for developing caries.

Whereas Tables 3 and 4 explained descriptive analysis of genotype of hBD1 SNPs between study and control groups according to caries experience (DMFT/DMFS) and caries severity, there is no significant difference among genotypes of each SNP in both groups. In high caries group, the genotype of rs5743418 showed significant difference ($P = 0.006$) with the DMFT.

By using spearman's coefficient correlation (r), these results showed non-significant correlations between hBD1 and caries experience as demonstrated in Table 5.

The correlation between hBD1and caries severity in high caries group revealed a negative significant correlation in rs11362 ($r = –0.453$, $P = 0.004$) as shown in Table 6.

Salivary hBD1 assessment

There are high concentration levels of salivary hBD1 in control group compared to study group with significant difference ($P = 0.038$) as explained in Table 7.

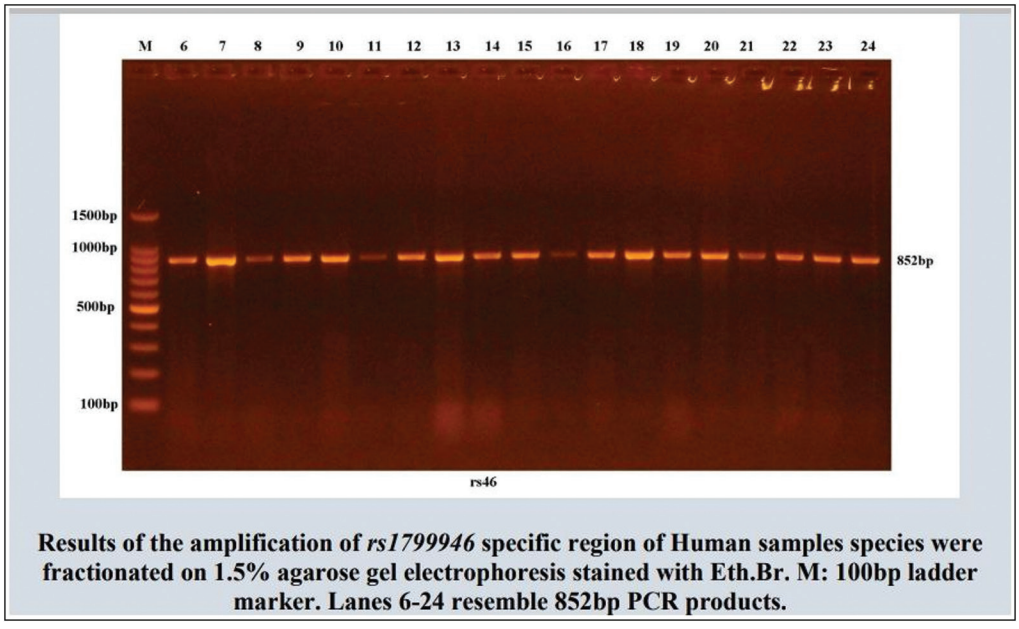

Figure 1: Electrophoresis of PCR products for *DEFB1* gene

Table 1: Genotype frequency comparisons calculated of hBD1 SNPs

rs5743418 (C > T) Genotypes frequency		Study groups		OR	CI	RR	χ^2	*P*-value
		High caries $n = 39$	Low caries $n = 39$					
Homozygous	CC	27 (69.2%)	36 (92.3%)	0.19	(0.04–0.73)	0.75	1.28	0.256 NS
Heterozygous	CT	10 (25.6%)	3 (7.6%)	5.33	(1.04–16.4)	1.33	3.77	0.050*
Homozygous	TT	2 (5.1%)	0 (0.0%)	–	–	–	0.591	0.702 NS
HWE χ^2	0.653	0.062						
P value	0.721NS	0.969NS						
rs1799946 (G > A								
Homozygous	GG	6 (15.3%)	7 (17.9%)	0.83	(0.25–2.74)	0.86	0.076	0.781 NS
Heterozygous	GA	23 (58.9%)	27 (69.2%)	0.63	(0.25–1.62)	1.17	0.320	0.571 NS
Homozygous	AA	10 (25.6%)	5 (12.8%)	2.34	(0.71–7.64)	2.0	1.67	0.196 NS
HWE χ^2	1.438	5.879						
P value	0.487NS	0.052NS						
rs11362 (G > A)								
Homozygous	GG	15 (38.4%)	15 (38.4%)	1.0	(0.40–2.49)	1.0	0.00	1.00 NS
Heterozygous	AG	24 (61.5%)	22 (56.4%)	1.24	(0.50–3.05)	1.09	0.078	0.768 NS
Homozygous	AA	0 (0.0%)	2 (5.13%)	–	–	–	0.259	0.682 NS
HWE χ^2	7.703	2.826						
P value	0.021NS	0.243NS						

OR: odds ratio, CI: confidence interval, RR: relative risk, χ^2: chi-square, *P* value: probability value, HWE: Hardy-Weinberg equilibrium, NS: non-significant

* Significant

Table 2: Allele frequency comparisons calculated of hBD1 SNPs

Allele frequency rs5743418	High caries $n = 39$	Low caries $n = 39$	χ^2	(*P* value)
C	64 (82.0%)	75 (96.1%)	7.988	0.004*
T	14 (17.9%)	3 (3.5%)		
HWE (*P* value)	0.418NS	0.802NS		
rs1799946				
G	35 (44.8%)	41 (52.5%)	0.923	0.336NS
A	43 (55.1%)	37 (47.4%)		
HWE (*P* value)	0.230NS	0.015*		
rs11362				
G	54 (69.2%)	52 (66.6%)	0.117	0.731NS
A	24 (30.7%)	26 (33.3%)		
HWE (*P* value)	0.005*	0.092NS		

χ^2: Chi-square, *P* value: probability value, HWE: Hardy-Weinberg equilibrium, NS: non-significant,

* Significant

The salivary hBD1 levels mean in low and high caries activity groups according to genotype of hBD1 SNPs. The B-Defensin 1 mean was higher in low caries group than high caries group in TT and AA genotype. There was no significant difference among genotype of each SNPs in both groups as described in Table 8.

Discussion

The development of caries is a complex trait influenced by both genetic and environmental factors that act synergistically to contribute to the pathology. Among the genetic factors, variants in genes encoding proteins involved in the innate immunity, which serve as the first line of defense against pathogens associated with caries

Table 3: The genotypes of hBD1 SNPs among study and control groups according to caries experience (DMFT/DMFS)

Group	SNP	Index	Genotype						Statistics	*P* value
			Homozygote		Heterozygote		Rare Homozygote			
			Mean	SE	Mean	SE	Mean	SE		
Low	rs5743418	DS	0.889	0.173	1.333	0.667			0.503	0.483
		DMFS	1.111	0.217	1.333	0.667			0.081	0.777
		DMFT	0.861	0.144	1.333	0.667			0.789	0.380
	rs1799946	DS	0.800	0.490	1.143	0.340	0.889	0.209	0.199	0.821
		DMFS	0.800	0.490	2.143	0.670	0.926	0.206	2.977	0.064
		DMFT	0.800	0.490	1.286	0.286	0.815	0.169	0.819	0.449
	rs11362	DS	0.667	0.232	1.000	1.000	1.091	0.236	0.744	0.482
		DMFS	0.667	0.232	2.000	2.000	1.364	0.283	1.893	0.165
		DMFT	0.667	0.232	1.000	1.000	1.045	0.180	0.828	0.445
High	rs5743418	DS	15.037	0.871	18.400	1.979			3.263	0.079
		DMFS	17.667	0.820	21.200	1.960			3.900	0.056
		DMFT	10.370	0.214	12.500	1.057			8.723	0.006
	rs1799946	DS	17.700	1.647	18.167	2.651	14.913	0.951	1.649	0.207
		DMFS	19.700	1.687	18.167	2.651	18.348	0.925	0.290	0.750
		DMFT	11.300	1.065	10.000	0.516	10.957	0.317	0.730	0.489
	rs11362	DS	16.867	1.245			15.667	1.091	0.500	0.484
		DMFS	19.667	1.256			18.042	1.006	1.013	0.321
		DMFT	10.933	0.733			10.875	0.315	0.007	0.934

DS: decay surface, DMFT: decayed, missing, filled, permanent tooth, DMFS: decayed, missing, filled permanent tooth surface, SE: stander error, *P* value: probability value

Table 4: The genotypes of hBD1 SNPs among study and control groups according to caries severity

Group	SNP	Index	Genotype						Statistics	*P* value
			Homozygote		Heterozygote		Rare homozygote			
			Mean	SE	Mean	SE	Mean	SE		
Low	rs5743418	D1	8.806	0.732	10.000	3.606			0.194	0.662
		D2	0.889	0.173	1.333	0.667			0.503	0.483
		D3	0.000	0.000	0.000	0.000			–	–
		D4	0.000	0.000	0.000	0.000			–	–
	rs1799946	D1	9.000	2.121	7.857	0.986	9.148	0.938	0.224	0.800
		D2	0.800	0.490	1.143	0.340	0.889	0.209	0.199	0.821
		D3	0.000	0.000	0.000	0.000	0.000	0.000	–	–
		D4	0.000	0.000	0.000	0.000	0.000	0.000	–	–
	rs11362	D1	9.333	1.214	7.000	3.000	8.773	0.953	0.250	0.780
		D2	0.667	0.232	1.000	1.000	1.091	0.236	0.744	0.482
		D3	0.000	0.000	0.000	0.000	0.000	0.000	–	–
		D4	0.000	0.000	0.000	0.000	0.000	0.000	–	–
High	rs5743418	D1	7.037	0.689	4.700	0.870			3.478	0.071
		D2	9.778	0.510	12.500	2.001			3.485	0.070
		D3	2.333	0.338	2.300	0.633			0.002	0.961
		D4	2.926	0.837	3.600	1.815			0.147	0.704
	rs1799946	D1	6.300	1.136	7.500	1.928	6.522	0.705	0.221	0.803
		D2	12.600	1.893	9.833	0.910	10.130	0.678	1.492	0.239
		D3	2.000	0.615	2.000	0.931	2.391	0.349	0.214	0.808
		D4	3.100	1.038	6.333	2.716	2.391	0.897	1.836	0.174
	rs11362	D1	6.867	0.850			6.458	0.775	0.118	0.733
		D2	11.800	1.377			10.042	0.603	1.762	0.193
		D3	2.400	0.524			2.125	0.342	0.211	0.648
		D4	2.667	0.893			3.500	1.065	0.299	0.588

D(1-4): decay severity scores, SE: stander error, *P* value: probability value

Table 5: Spearman's correlation between hBD1and caries experience in study groups

Groups		DS		DMFS		DMFT	
		rsp	*P*	Rsp	*P*	Rsp	*P*
Low	rs5743418	0.100	0.545	0.053	0.747	0.047	0.777
	rs1799946	–0.017	0.919	0.010	0.952	0.020	0.903
	rs11362	–0.020	0.902	0.012	0.940	–0.025	0.879
High		rsp	*P*	Rsp	*P*	Rsp	*P*
	rs5743418	–0.086	0.603	0.159	0.334	0.228	0.162
	rs1799946	–0.107	0.515	–0.041	0.806	0.004	0.978
	rs11362	0.092	0.577	0.094	0.568	–0.025	0.880

DS: decay surface, DMFT: decayed, missing, filled, permanent tooth, DMFS: decayed, missing, filled permanent tooth surface, rsp: spearman's coefficient correlation, *P* value: probability value

Table 6: Spearman's correlation between hBD1and caries severity in study groups

Groups		D1		D2		D3		D4	
		rsp	*P*	rsp	*P*	rsp	*P*	rsp	*P*
	rs5743418	0.143	0.385	0.100	0.545				
	rs1799946	–0.138	0.403	–0.017	0.919				
	rs11362	0.019	0.910	–0.020	0.902				
High		rsp	*P*	rsp	*P*	rsp	*P*	rsp	*P*
	rs5743418	0.167	0.308	0.000	10.000	0.235	0.150	–0.284	0.080
	rs1799946	0.016	0.921	–0.138	0.401	0.236	0.147	–0.185	0.258
	rs11362	0.177	0.280	0.116	0.483	–0.453	0.004	0.191	0.245

D(1-4): decay severity scores, rsp: spearman's coefficient correlation; *P* value: probability value

Table 7: Explained the salivary hBD1 levels in study and control groups

	Groups		Wilcoxon sum rank test	*P* value
	Low	High		
Mean	570.773	294.957	2.135	0.038
±SE	118.691	50.984		
Median	276.9430	134.4530		
Mean rank	43.99	35.01		

SE: stander error, *P* value: probability value

Table 8: The salivary hBD1 mean in low and high caries activity groups according to genotype of hBD1 SNPs

Group	SNP	Genotype						Statistics	*P* value
		Homozygote		Heterozygote		Rare homozygote			
		Mean	SE	Mean	SE	Mean	SE		
Low	rs5743418	591.306	127.882	324.387	111.328	0	0	0.353	0.556
	rs1799946	678.868	223.639	533.221	294.118	560.492	151.784	0.062	0.940
	rs11362	709.136	223.516	1219.486	103.946	417.461	119.617	1.541	0.228
High	rs5743418	299.637	63.638	231.070	63.860	0	0	0.374	0.545
	rs1799946	269.502	92.794	204.591	90.987	329.598	73.665	0.397	0.675
	rs11362	240.936	63.992	0	0	328.720	72.746	0.696	0.409

SE: stander error; *P* value: probability value

development, are hypothesized to play a crucial role in susceptibility to caries. hBD1, which is constitutively expressed in the mucosa, is a potential candidate for modifying host susceptibility to infections, and therefore influencing caries experience. However, other important factors such as consumption of acid drinks and sugar, frequency of toothbrushing, and the composition of the mucosal microbiota should also be taken into consideration, as highlighted by Navarra *et al.*[22] Our results should be interpreted in the context of caries being a multifactorial disease, where innate immunity is not the sole factor influencing susceptibility to caries experience. Other factors, such as polymorphisms in genes involved in enamel development, have been reported to be associated

with increased susceptibility to caries, as reported by Abbasoğlu *et al*.[23]

SNP (rs11362) has been previously identified as associated with DMFT in Navarra *et al*.[22] in Krasone *et al*.[24] Both of these independent studies reported that the presence of the G allele or GG genotype conferred a significant risk of caries, which is in contrast to the findings of this study where no association was found with DMFT, and the G allele and GA genotype had a higher frequency. These studies differed from the current study as their participants were sampled from genetically isolated villages, which is not the case in Baghdad city. Isolated populations have smaller population sizes and decreased genetic variability, resulting in stronger effects of random genetic drift,[25] and providing more power for genetic association mapping investigations.[26] However, a study on a group of Turkish children by Abbasoğlu *et al*.[23] reported a lack of association between DEFB1 polymorphisms (rs11362 and rs1800972) and susceptibility to develop caries.

This study is in agreement with the findings of Polesello *et al*.,[27] who reported an association between DEFB1 (rs1799946), (rs1800972) SNPs, and hBD1 salivary concentrations, but not for (rs11362) SNP, in a small cohort of healthy subjects from north-eastern Italy. Navarra *et al*.[22] also revealed a positive association between DMFT and DEFB1 -52G>A (rs1799946) SNP. This SNP has been widely cited in the literature for its correlation with various pathologies such as chronic tonsillitis, diabetes, HIV infection, Crohn disease, Staphylococcus aureus infection, contact lens keratitis, atopic dermatitis, and periodontitis. Furthermore, this SNP has recently been implicated in the list of genetic variants predisposing to neonatal sepsis,[28] further highlighting the importance of this DEFB1 5'-untranslated region functional polymorphism in the context of infections. According to Polesello *et al*.,[27] carriers of the DEFB1 -52G/G genotype are able to produce higher levels of hBD1, potentially providing increased protection against infections, and consequently against DMFT.

Taking all of these factors into consideration, our findings support the significance of innate immunity in the development of dental caries, indicating that these polymorphisms could serve as potential markers for assessing an individual's risk of developing caries. However, it is important to bear in mind that caries experience is a multifactorial disease influenced by the synergistic action of various factors. Genetic studies that identify novel potential candidates for caries susceptibility could be valuable in designing a chip that contains the most impactful genetic variations, which could be used as a screening tool in dental clinical practice.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References
1. Juma Z, Raghad F, Yasameen A. Correlation between caries related microorganisms in the dental plaque and saliva with dental caries level in the upper and lower jaws in 5-9 years old children in Baghdad city. J Bagh Coll Dentistry 2016;28:132-6.
2. Aziz A, Taha A. The salivary inflammatory biomarkers (interleukin-6, C-reactive protein) in relation with caries-experience among a group of 12-year-old obese boys. J Bagh Coll Dentistry 2016;28:138-42.
3. Vieira AR. Genetic basis of dental caries and periapical pathology. In: Genetic Basis of Oral Health Conditions. Switzerland AG: Springer Nature; 2019. p. 33-42.
4. Al-zahraa JJ, Aldhaher ZA. Evaluation of mutans streptococci concerning oral health in the saliva of pregnant women. Revis Bionatura 2023;8:81.
5. Mohammed HA, Sahi NM, Ahmed RT, Al-Rubaye AF. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601-8.
6. Ali RA, Radeef SM, Mohammed NB, Diab BS. Oral health related quality of life among dental implant patients in relation to temporomandibular joint function. Med J Babylon 2022;19:609-14.
7. Slayton RL, Cooper ME, Marazita ML. Tuftelin, mutans streptococci, and dental caries susceptibility. J Dent Res 2005;84:711-4.
8. Andrade FB, Oliveira JC, Yoshie MT, Guimarães BM, Gonçalves RB, Schwarcz WD. Antimicrobial activity and synergism of lactoferrin and lysozyme against cariogenic microorganisms. Braz Dent J 2014;25:165-9.
9. Cogulu D, Onay H, Ozdemir Y, Aslan G I, Ozkinay F, Kutukculer N, *et al*. Associations of interleukin (IL)-1β, IL-1 receptor antagonist, and IL-10 with dental caries. J Oral Sci 2015;57:31-6.
10. Dale BA, Tao R, Kimball JR, Jurevic RJ. Oral antimicrobial peptides and biological control of caries. BMC Oral Health 2006;6:S13.
11. Van Nieuw Amerongen A, Bolscher JGM, Veerman ECI. Salivary proteins: protective and diagnostic value in cariology? Caries Res 2004;38:247-53.
12. Prokopovic V, Popovic M, Andjelkovic U, Marsavelski A, Raskovic B, Gavrovic-Jankulovic M, *et al*. Isolation, biochemical characterization and anti-bacterial activity of BPIFA2 protein. Arch Oral Biol 2014;59:302-9.
13. Yildiz G, Ermis RB, Calapoglu NS, Celik EU, Türel GY. Gene-environment interactions in the etiology of dental caries. J Dent Res 2016;95:74-9.
14. Giugliano D, d'Apuzzo F, Majorana A, Campus G, Nucci F, Flores-Mir C, *et al*. Influence of occlusal characteristics, food intake and oral hygiene habits on dental caries in adolescents: A cross-sectional study. Eur J Paediatr Dent 2018;19:95-100.
15. Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader M-J, Bramlett MD, *et al*. Influences on children's oral health: A conceptual model. Pediatrics 2007;120:e510-20.
16. Mahato NK, Pal GP. Genetics in Dentistry. New Delhi, India: Jaypee Brothers Medical Publishers Pvt. Limited; 2010.
17. Fadhil R, Akram HM, Najah A, Gul SS. Association of matrix metalloproteinase-1-1607 1G/2G single nucleotide polymorphism genotypes with periodontitis in Iraqi population. Braz Dent Sci 2022;25:e3283.
18. Ghufran AA, Jafar Z, AL-Ghurabi B. The relation of salivary cathelicidin and beta-defensin with dental caries of schoolchildren. J Res Med Dent Sci 2021;9:30-5.
19. Vieira AR, Marazita ML, Goldstein-McHenry T. Genome-wide scan finds suggestive caries loci. J Dent Res 2008;87:435-9.
20. World Health Organization. Oral Health Surveys Basic Methods. 5th ed. Geneva: WHO Press; 2013. Available from: www.who.int/oral_health.

21. Muhlemann HR. Oral epidemiology-caries. In: Zeitschriften-Verlag B, Quintessenze D, editors. Introduction to Oral Preventive Medicine; 1976. p. 73-100.
22. Navarra Chiara O, Robino A, Pirastu N, Bevilacqua L, Gasparini P, Di Lenarda R, *et al*. Caries and innate immunity: DEFB1 gene polymorphisms and caries susceptibility in genetic isolates from North-Eastern Italy. Caries Res 2016;50:589-94.
23. Abbasoğlu Z, Tanboğa I, Küchler EC, Deeley K, Weber M, Kaspar C, *et al*. Early childhood caries is associated with genetic variants in enamel formation and immune response genes. Caries Res 2015;49:70-7.
24. Krasone K, Lāce B, Akota I, Care R, Deeley K, Küchler EC, *et al*. Genetic variation in the promoter region of beta-defensin 1 (DEFB 1) is associated with high caries experience in children born with cleft lip and palate. Acta Odontol Scand 2014;72:235-40.
25. Kristiansson K, Naukkarinen J, Peltonen L. Isolated populations and complex disease gene identification. Genome Biol 2008;9:109.
26. Peltonen L, Jalanko A, Varilo T. Molecular genetics of the Finnish disease heritage. Hum Mol Genet 1999;8:1913-23.
27. Polesello V, Zupin L, Di Lenarda R, Biasotto M, Ottaviani G, Gobbo M, *et al*. Impact of DEFB1 gene regulatory polymorphisms on hBD-1 salivary concentration. Arch Oral Biol 2015;60: 1054-8.
28. Esposito S, Zampiero A, Pugni L, Tabano S, Pelucchi C, Ghirardi B, *et al*. Genetic polymorphisms and sepsis in premature neonates. PLoS One 2014;9:e101248.