Original Article

Evaluation of Single Nucleotide Polymorphisms of Salivary Lactoferrin 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: As human genomics advances, increased attention is directed toward identifying genetic factors linked to dental caries. Among the various multifunctional proteins present on mucosal surfaces in the body, lactoferrin (LTF) stands out. This protein plays a role in regulating immune-inflammatory and antibacterial responses. While a genetic role in caries susceptibility has been acknowledged, the specific genetic components contributing to this susceptibility remain largely unexplored. **Objectives:** This study aimed to explore the connection between genetics and the level of salivary LTF in relation to caries susceptibility among adolescents in Baghdad City. **Materials and Methods:** A case–control study encompassing 78 adolescents (aged 13–15 years) was conducted. This group was divided into 39 subjects with very low caries (control group) and 39 subjects with high caries (study group) in Baghdad City. Unstimulated saliva samples were collected, and dental caries were assessed using the DMFS/DMFT (decayed, missing, filled surface/teeth) index. Subsequent to DNA extraction, single nucleotide polymorphisms (SNPs) were genotyped through polymerase chain reaction and DNA sequencing. Four specific SNPs were identified: rs6441989, rs34265215, rs77648833, and rs35883833. The correlation between these SNPs and dental caries was measured using odds ratios along with a 95% confidence interval. **Results:** The concentration of salivary LTF was significantly higher in the control group (low caries) than in the study group (high caries) ($P = 0.01$). However, no significant differences were observed between the study and control groups in the genotypes. Additionally, no significant deviation from the Hardy–Weinberg equilibrium was noted. **Conclusion:** The level of salivary LTF did influence caries susceptibility. However, at the genetic level, the investigated SNPs did not demonstrate any discernible association with dental caries.

Keywords: Allele, dental caries, genetics, health risk, lactoferrin, single nucleotide polymorphisms

Introduction

Dental caries poses a significant public health challenge and is considered the most prevalent infectious oral condition in humans. It is a complex disease influenced by numerous factors, including lifestyle, obesity, socioeconomic status, pregnancy, genetic factors, and the attributes of the oral environment.[1-4] It results in progressive tooth tissue destruction, inflammation of the dental pulp and periapical tissues, and subsequent pain, infection, and tooth loss, and expended a lot on its treatment each year. This can also have a detrimental impact on various aspects of individuals' lives, including difficulties with learning, eating, and sleeping.[5] Despite the advancement of methods for prevention and treatment, dental caries remain a significant concern, impacting the quality of life.[6-8]

In 2001, the National Institutes of Health Consensus Development Program outlined six major clinical caries research directions, one of which emphasized the importance of genetic studies in identifying genes and genetic markers with diagnostic, prognostic, and therapeutic value.[9]

Access this article online

Quick Response Code:

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

DOI: 10.4103/MJBL.MJBL_1304_23

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

Submission: 29-Aug-2023 **Accepted:** 11-Dec-2023 **Published:** 30-Apr-2026

How to cite this article: Alidan EA, Mohammed AT. Evaluation of single nucleotide polymorphisms of salivary lactoferrin in relation to dental caries. Med J Babylon 2026;23:452-9.

Over the past 2 decades, there has been a significant increase in research efforts focused on investigating the role of genetic factors in individual susceptibility to dental caries. Extensive endeavors in gene mapping have been undertaken to pinpoint distinct genetic locations that contribute to the susceptibility to dental caries.[10] Findings from prior research have illuminated characteristics associated with processes linked to caries development, categorized into the following four groups: salivary composition and flow, tooth morphology, dietary and taste preferences, and enamel and dentin formation. Saliva contains elements that possess the capability to directly combat cariogenic bacteria. Additionally, it is abundant in calcium and phosphates, which play an active role in the remineralization of tooth enamel. The mechanical action of saliva flow aids in dislodging pathogens such as viruses, bacteria, and yeast from both teeth and mucosal surfaces. Moreover, saliva has the ability to aggregate microbes, facilitating their removal through swallowing before they establish firm attachment.[11]

Lactotransferrin (LTF) is a versatile metalloprotein categorized within the transferrin family, possessing a molecular weight of 80 kDa and consisting of 690 amino acids.[12] Regarded as a notable component of the oral immune system, LTF functions as a secretory molecule within salivary fluid.[13] LTF is synthesized in various tissues and is found in a range of bodily fluids across different organisms, including saliva, tears, semen, sweat, colostrum, milk, and nasal secretions.[14] Polymorphonuclear leukocytes harbor a substantial quantity of LTF, recognized as a cytokine with a role in safeguarding against various infections.[12] Its antimicrobial attributes are especially noteworthy in human infants, as it acts by chelating iron, thereby depriving microorganisms of their vital element.[15] LTF has the capability to dampen the innate immune response activated by lipopolysaccharides, regulate the adaptive immune system,[16] and significantly contribute to physiological balance. This equilibrium is closely linked to the onset of diseases, thereby endowing LTF with a broad spectrum of antimicrobial, immunoregulatory, and anti-inflammatory properties.[17-19]

A significant correlation was identified between the presence of decayed surfaces and the concentration of salivary LTF.[20] Despite the acknowledged antibacterial attributes of LTF, individuals with lower DMFT scores exhibited diminished LTF expression in their saliva. This could potentially be attributed to the possibility that an elevated LTF production serves as an effort to regulate the disease process.[12,21]

In Iraq, research has been conducted to establish connections between genetic factors and other diseases like periodontal disease and bacteria-causing caries.[22,23] However, no prior studies have been able to establish links between genes related to antimicrobial properties, such as lactoferrin (LTF), in the saliva of individuals with dental caries. As a result, the primary objective of the present study was to identify and assess individuals susceptible to dental caries while comprehending the role of genes in the development of this condition. The study encompassed examinations aimed at determining whether variations in the LTF gene were linked to the occurrence of caries.

Materials and Methods

In this particular case–control study, a group of 78 healthy and unrelated Iraqi adolescents was included. Among them, 39 subjects were selected for the study group due to having a high prevalence of caries, while another 39 were designated as the control group due to having very few instances of caries. All participants had complete permanent dentition and lacked any dental anomalies. Prior to the examination, consent was obtained from the parents of the patients.

The inclusion criteria encompassed individuals aged between 13 and 15 years who were part of the Iraqi Arab Population. They should not have had any systemic diseases or taken any medications in the preceding 3 weeks. The study group required a high caries score with a DMFT (decayed, missing, filled teeth) value greater than 9, whereas the control group should have had a low caries score with a DMFT value less than 2.[24]

Exclusion criteria involved patients with systemic diseases, cleft lips, congenital anomalies, widespread dental issues, or those who were using fixed orthodontic appliances. Participants who had received fluoride supplements or had undergone fissure sealant treatment were also excluded. Individuals falling within the DMFT range of more than 2 but less than 9 were not considered for the study.

Various demographic details, such as age and gender, along with medical and dental histories, were recorded. Unstimulated saliva was collected, followed by an intraoral examination using disposable instruments. The evaluation of tooth decay was scored based on the criteria set by the World Health Organization,[25] utilizing the "decayed missing and filled teeth/surfaces" (DMFS/DMFT) index for permanent teeth, without the use of radiographs. The severity of dental caries was assessed according to WHO's 1976 guidelines.[26]

Unstimulated saliva was gathered over a span of 5 min. Participants were instructed to expel saliva through a funnel into a sterilized tube that featured a scale, and this was repeated every minute, following the methodology outlined by Navazesh in 2008.[27] After the completion of saliva collection, the samples were divided into two segments. In the case of the portion meant for determining

LTF concentration, it was subjected to centrifugation at 4000 revolutions per minute (rpm) for a duration of 5 min. This process was undertaken to segregate the mucins. Subsequently, the clear supernatant was separated using a micropipette and was then stored at approximately -80°C, as per the manufacturer's instructions (MyBioSource, USA). For the other portion of the sample intended for DNA extraction, centrifugation was performed at a speed of 12,000 revolutions per minute (rpm) for a span of 3 min.

DNA extraction and quantitation

The genomic DNA was extracted from the saliva samples using the ReliaPrep™ Saliva gDNA Miniprep System protocol by Promega. The Quantus Fluorometer was employed to determine the concentration of the extracted DNA and assess the quality of the samples for subsequent procedures.

Primer preparation, optimization, and polymerase chain reaction (PCR) amplification

The primers were provided by Macrogen Company in a lyophilized (freeze-dried) state. The DNA template was subjected to amplification using the same pair of primers, consisting of a forward primer and a reverse primer. Following the PCR amplification process, agarose gel electrophoresis was utilized to verify the successful amplification. The efficacy of the PCR was entirely reliant on the quality of the extracted DNA.

Standard sequencing

The PCR products were submitted for Sanger sequencing using the ABI3730XL automated DNA sequencer from Macrogen Corporation—Korea. Subsequently, the outcomes were delivered via email and subsequently assessed using the Geneious software. DNA Concentration range was 12–20 ng/µl.

ELISA assay for measuring LTF levels

The collected saliva samples from participants were examined for LTF concentration using commercially available ELISA kits in accordance with the manufacturer's instructions (MyBioSource, USA).

Statistics

A computerized software statistical package for social science (SPSS version-24) was used. The variation of frequencies between groups was analyzed using the Chi-square test. Hardy–Weinberg equilibrium (HWE) was used to calculate the expected common homozygotes, expected heterozygotes, and expected rare homozygotes. The 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% CIs). This indicates how many times more frequently a disease develops in individuals carrying the allele or genotype than in individuals lacking it. The descriptive analysis used was mean, standard deviation (SD), and standard error (SE) for the quantitative variable. Inferential analysis used was Spearman correlation, which tests the monotonic correlation for qualitative and non-parametric variables, and one-way analysis of variance, which tests the difference between k-independent groups.

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 the patient's verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee of Baghdad University, College of Dentistry, according to document number 366 (1/8/2021) to get this approval.

Results

Genetic analysis of LTF single nucleotide polymorphisms (SNPs)

Agarose gel electrophoresis of PCR products

The genetic analysis was done for LTF SNPs gene polymorphisms, and four polymorphisms (rs6441989, rs34265215, rs77648833, and rs35883833) were detected

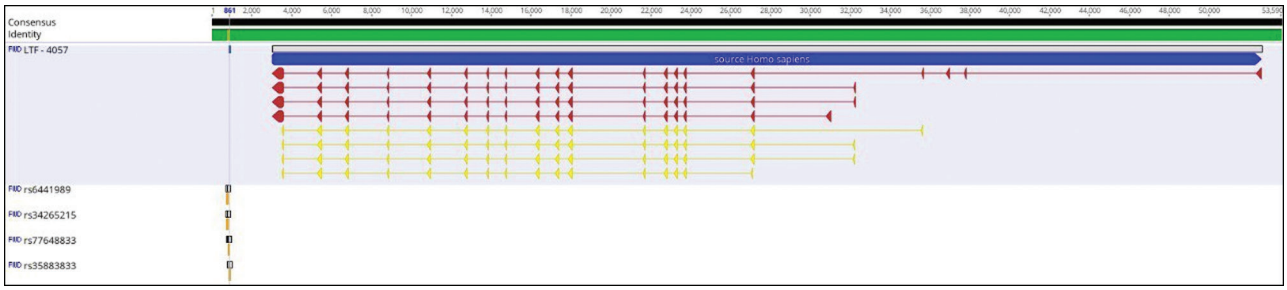

Figure 1: Location of lactoferrin SNPs gene

located at the end of a gene, as in Figure 1. According to primer design, which was higher at 768bp, the result of PCR was shown in Figure 2.

LTF gene sequencing

Sequencing of the LTF gene in the present study resulted in the detection of four SNPs (rs6441989, rs34265215, rs77648833, and rs35883833) in both groups, as shown in Figure 3.

Genotype and allele frequency analysis for LTF gene

Table 1 shows the genotype and allele frequency comparisons calculated for LTF gene SNPs, and it showed no significant difference among genotypes in the study and control groups and no significant deviation from HWE.

Table 2 demonstrates the frequency of the major alleles was higher in the low caries group than in high caries in all SNPs except for **rs77648833**. A significant difference was not found between high and low caries groups in all SNPs, and no significant deviation from HWE.

Correlation of LTF gene SNPs with different parameters

By using Spearman's correlation, the current results of the correlations among LTF gene SNPs and caries experience and caries severity in the low and high groups revealed nonsignificant correlations, as shown in Tables 3 and 4.

Salivary LTF assessment

Table 5 describes the salivary LTF concentrations in low and high caries activity groups. It was found that the

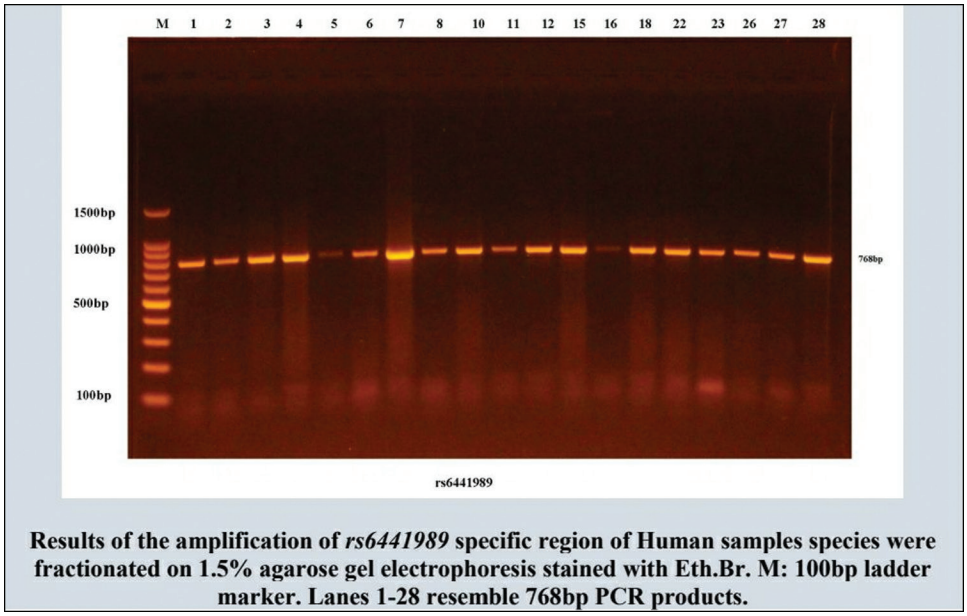

Figure 2: Electrophoresis of PCR products for lactoferrin gene

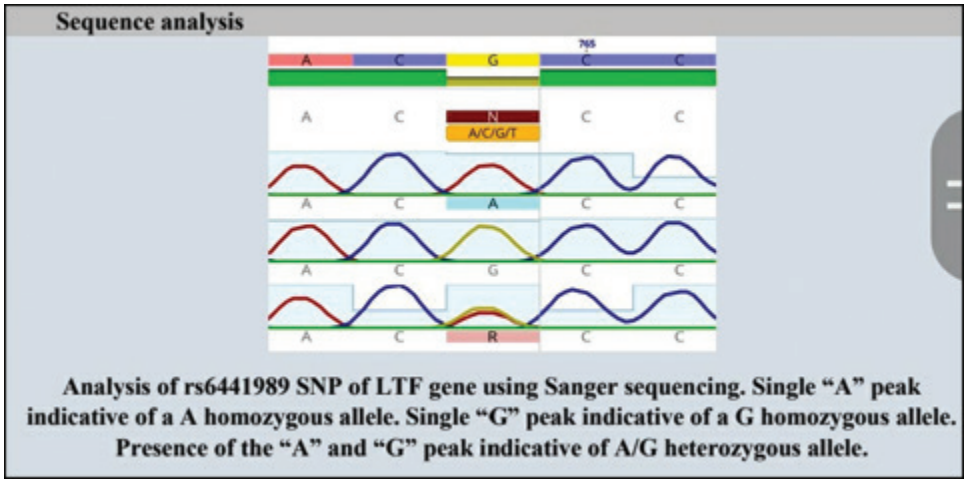

Figure 3: The sequence lactoferrin gene

Table 1: Genotype and allele frequency comparisons calculated for lactoferrin gene SNPs

rs6441989 (A > G) Genotypes frequency		Study groups		OR	CI	RR	χ^2	(*P*-value)
		High caries *n* = 39	Low caries *n* = 39					
Homozygous	AA	6 (15.3%)	7 (17.9%)	0.83	(0.25–2.74)	0.86	0.076	0.781 NS
Heterozygous	AG	20 (51.2%)	21 (53.8%)	1.20	(0.37–2.19)	1.17	0.024	0.875 NS
Homozygous	GG	13 (33.3%)	11 (28.2%)	1.27	(0.48–3.33)	1.18	0.167	0.683 NS
HWE χ^2	0.139	0.304						
P-value	0.932NS	0.858NS						
rs34265215 (C > A)								
Homozygous	CC	33 (84.6%)	34 (87.1%)	0.81	(0.22–2.90)	0.97	0.014	0.902 NS
Heterozygous	CA	5 (12.82%)	5 (12.82%)	1.24	(0.26–3.77)	1.03	0.00	1.000 NS
Homozygous	AA	1 (2.56%)	0 (0.0%)	–	–	–	0.041	0.915 NS
HWE χ^2	0.807	0.182						
P-value	0.405NS	0.912NS						
rs77648833 (A > G)								
Homozygous	AA	35 (89.7%)	31 (79.4%)	2.26	(0.61–8.23)	1.33	0.242	0.623 NS
Heterozygous	AG	4 (10.26%)	8 (20.51%)	0.44	(0.12–1.61)	0.89	1.33	0.248 NS
Homozygous	GG	0 (0.0%)	0 (0.0%)	–	–	–	0.00	1.000 NS
HWE χ^2	0.113	0.509						
P-value	0.944NS	0.775NS						
rs35883833 (C > T)								
Homozygous	CC	33 (84.6%)	34 (87.1%)	0.81	(0.22–2.90)	0.97	0.014	0.902 NS
Heterozygous	CT	5 (12.82%)	5 (12.82%)	1.24	(0.26–3.77)	1.03	0.000	1.000 NS
Homozygous	TT	1 (2.56%)	0 (0.0%)	–	–	–	0.041	0.915 NS
HWE χ^2	0.807	0.182						
P-value	0.405NS	0.912NS						

Abbreviations: OR. odds ratio; CI, confidence interval; RR, relative risk; χ^2, chai-square; *P*-value, probability value; HWE, Hardy–Weinberg equilibrium; NS, nonsignificant

Table 2: Allele frequency comparisons calculated of lactoferrin gene SNPs

Allele frequency rs6441989 (A > G)	High caries *n* = 39	Low caries *n* = 39	χ^2	*P*-value
A	32 (41.0%)	35 (44.8%)	0.235	0.627NS
G	46 (58.9%)	43 (55.1%)		
HWE (*P*-value)	0.708NS	0.581NS		
rs34265215 (C > A)				
C	71 (91.0%)	73 (93.5%)	0.361	0.547NS
A	7 (8.9%)	5 (6.4%)		
HWE (*P*-value)	0.178NS	0.668NS		
rs77648833 (A > G)				
A	74 (94.8%)	70 (89.7%)	1.444	0.229NS
G	4 (5.1%)	8 (10.2%)		
HWE (*P*-value)	0.735NS	0.474NS		
rs35883833 (C > T)				
C	71 (91.0%)	73 (93.5%)	0.361	0.547NS
T	7 (8.9%)	5 (6.4%)		
HWE (*P*-value)	0.178NS	0.668NS		

Abbreviations: χ^2, chai- square; *P*-value, probability value; HWE, Hardy–Weinberg equilibrium; NS, nonsignificant

salivary LTF in the low caries activity group was higher than in the high caries activity group, with a significant difference between them.

The findings of the present study revealed that there is a negative, weak, significant correlation between rs1347617470 and salivary LTF levels at (r = -0.398; P = 0.012) in the control group only. On the other hand, the study could not detect any significant correlations regarding the remaining LTF SNPs and their concentration [Table 6].

Table 7 shows the salivary LTF concentrations mean in low and high caries activity groups according to the genotype

Table 3: Spearman's correlation between lactoferrin SNPs and caries experience in study groups

Groups		DS		DMFS		DMFT	
		Rsp	*P*	Rsp	*P*	Rsp	*P*
Low	rs6441989	0.020	0.902	0.096	0.559	0.068	0.682
	rs34265215	-0.014–	0.933	-0.067–	0.686	-0.031–	0.850
	rs77648833	0.033	0.843	0.232	0.156	0.137	0.406
	rs35883833	-0.014–	0.933	-0.067–	0.686	-0.031–	0.850
High	rs6441989	-0.058–	0.726	-0.056–	0.734	-0.192–	0.241
	rs34265215	-0.130–	0.430	-0.219–	0.181	-0.098–	0.553
	rs77648833	0.102	0.537	0.025	0.878	-0.246–	0.131
	rs35883833	-0.130–	0.430	-0.219–	0.181	-0.098–	0.553

Abbreviations: 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 4: Spearman's correlation between lactoferrin gene SNPs and caries severity in study groups

Groups		D1		D2		D3		D4	
		Rsp	*P*	rsp	*P*	Rsp	*P*	Rsp	*P*
Low	rs6441989Lac	-0.103-	0.533	0.020	0.902				
	rs34265215Lac	-0.085–	0.608	-0.014–	0.933				
	rs77648833Lac	0.057	0.733	0.033	0.843				
	rs35883833Lac	-0.085–	0.608	-0.014–	0.933				
High	rs6441989Lac	-0.202–	0.218	-0.140–	0.396	-0.041–	0.805	0.119	0.472
	rs34265215Lac	-0.014–	0.934	-0.014–	0.934	-0.166–	0.312	0.000	0.999
	rs77648833Lac	-0.190–	0.247	-0.145–	0.380	-0.100–	0.543	0.294	0.069
	rs35883833Lac	-0.014–	0.934	-0.014–	0.934	-0.166–	0.312	0.000	0.999

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

Table 5: Salivary lactoferrin concentrations among low and high caries groups

	Groups		*T*-test	*P*-value
	Low	High		
Mean	3.442	2.017	2.613	0.011
±SD	3.003	1.603		

Abbreviations: SD, standard deviation; SE, standard error; *P*-value probability value

Table 6: Correlation of lactoferrin gene SNPs with lactoferrin levels in study and control groups

Groups		Lactoferrin	
		Rsp	*P*
Low	rs6441989Lac	0.154	0.350
	rs34265215Lac	-0.165–	0.314
	rs77648833Lac	-0.398–	0.012
	rs35883833Lac	-0.165–	0.314
High	rs6441989Lac	-0.045–	0.784
	rs34265215Lac	-0.075–	0.650
	rs77648833Lac	0.068	0.682
	rs35883833Lac	-0.075–	0.650

Abbreviations: rsp, Spearman's coefficient correlation; *P*-value, probability value

of LTF SNP. The LTF concentration mean was higher in the low caries group than in the high caries group in the AA genotype, with no significant difference among the genotypes of each SNP in both groups.

Discussion

The exploration of the mechanisms that contribute to an individual's susceptibility to caries aligns with the emergence of viable methods for comprehending genetic

Table 7: Salivary lactoferrin level among low and high caries groups according to genotype of lactoferrin SNP

Groups	SNPs	AA			GG			AG			Statistics	*P* value
		N	Mean	SE	*N*	Mean	SE	*N*	Mean	SE		
Low	rs34265215	34	3.717	0.534	5	1.567	0.336	0	0	0	2.311	0.137
	rs35883833	34	3.717	0.534	5	1.567	0.336	0	0	0	2.311	0.137
	rs6441989	7	2.243	0.791	11	4.698	1.103	21	3.183	0.605	1.653	0.206
	rs77648833	31	3.494	0.505	8	3.240	1.368	0	0	0	0.044	0.835
High	rs34265215	33	1.973	0.287	5	2.405	0.695	1	1.538	0.000	0.195	0.824
	rs35883833	33	1.973	0.287	5	2.405	0.695	1	1.538	0.000	0.195	0.824
	rs6441989	6	2.793	1.151	13	1.690	0.312	20	1.998	0.315	0.973	0.388
	rs77648833	35	1.896	0.216	4	3.078	1.763	0	0	0	2.004	0.165

Abbreviations: SE, standard error; *P*-value, probability value

predisposition to intricate human ailments. This is notably facilitated by tools brought forth through initiatives like the Human Genome Project. Multiple factors, including tooth morphology, buffering capacity, salivary flow, dietary practices, oral microbiome composition, oral hygiene practices, and previous occurrences of dental caries, undoubtedly exert significant influence on the formation of carious lesions.[17,28]

Extensive research has been conducted on the components and characteristics of saliva, yet certain aspects remain to be elucidated. Findings pertaining to salivary proteins, including LTF, have exhibited inconsistencies. This particular study aimed to examine the correlation between various SNPs within LTF and the vulnerability to dental caries. However, no statistically significant distinction in the pattern of polymorphisms was observed between the groups with low and high occurrences of dental caries in this investigation. This observation might be attributed to the complexity of polymorphism studies involving salivary proteins. In essence, a single polymorphism within the gene of a defense protein might not suffice to fully expound the intricacies of a multifactorial condition like dental caries.

Saliva contains numerous defense proteins, allowing compensatory mechanisms to counterbalance minor deficiencies effectively. Moreover, the substantial impact of oral hygiene, lifestyle choices, dietary habits, and the properties of saliva itself in relation to dental caries is so pronounced that a slight "defect" in LTF plays a negligible role in sustaining oral tissue health.

While LTF is indeed a captivating antimicrobial agent, its presence in saliva is just a minor aspect within the broader array of antimicrobial mechanisms. The examined SNPs did not exhibit any discernible connection between their polymorphisms and susceptibility to dental caries. Consequently, they were ruled out as risk factors for dental caries. Nonetheless, for a more precise validation of these findings, additional research is warranted. This entails conducting larger studies encompassing diverse ethnic groups and undertaking longitudinal investigations.
While LTF is indeed a captivating antimicrobial agent, its presence in saliva is just a minor aspect within the broader array of antimicrobial mechanisms. The examined SNPs did not exhibit any discernible connection between their polymorphisms and susceptibility to dental caries. Consequently, they were ruled out as risk factors for dental caries. Nonetheless, for a more precise validation of these findings, additional research is warranted. This entails conducting larger studies encompassing diverse ethnic groups and undertaking longitudinal investigations.

Variations in SNPs within the LTF gene might hold promise in mitigating the development of carious lesions. Nevertheless, the present study did not yield noteworthy correlations between the examined LTF SNPs and dental

caries. Interestingly, a contrasting outcome was reported by Doetzer *et al.*,[29] where they identified that the SNP rs6441989 was significantly less prevalent within the high caries group, suggesting a protective influence against caries occurrence.

When it comes to the SNPs rs34265215, rs77648833, and rs35883833, there have been no prior investigations into these specific variants. Additionally, these SNPs are not documented in ClinVar, which is a repository containing information about genomic variations and their implications for human health. Consequently, this study represents the initial endeavor in researching these SNPs and their potential associations.

A negative, weak, and statistically significant correlation was identified between rs1347617470 and the levels of salivary LTF in the low caries group. This correlation indicated that as the expression of this SNP increased, there was a corresponding decrease in the concentration of LTF in the saliva, which could be an indicator of a risk factor for caries occurrence.

As a result, this study is anticipated to serve as a stepping stone for forthcoming research endeavors encompassing a broader array of genes and genetic markers. Furthermore, it aims to inspire the initiation of regional genome-wide association studies focused on exploring the intricacies of dental caries within the Arab population and other ethnicity. Based on the findings presented in this report, there remains a need for additional research to authenticate and substantiate these results. This necessitates the incorporation of additional genetic variants to acquire a comprehensive comprehension of the contribution of genetic attributes in either rendering susceptibility to or conferring protection against dental caries.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References
1. Juma'a Z, Fadhil R, Abdul-Hussain Y. 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, Springer Nature Switzerland AG. 2019; 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. Gilchrist F, Marshman Z, Deery C, Rodd HD. The impact of dental caries on children and young people: What they have to say? Int J Paediatr Dent 2015;25:327-38.
6. Swadi AK, Jassim ZW, Kadum MU, Ali MRM. Influence of fluoride addition on hydroxyapatite prepared for medical applications. Baghdad Sci J [Internet] 2012;9:541-7.
7. 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. MJB 2022;19:601-8.
8. Ali RA, Radeef SM, Mohammed NB, Diab BS. Oral health related quality of life among dental implant patients in relation to temporomandibular joint function. MJB 2022;19:609-14.
9. NIH Consensus Statement. Diagnosis and management of dental caries throughout life. NIH Consens Statement 2001:1-24. http://consensus.nih.gov/ 2001/2001DentalCaries115html.htm.
10. Werneck R, Mira M, Trevilatto P. A critical review: An overview of genetic influence on dental caries. Oral Dis 2010;16:613-23.
11. Stookey GK. The effect of saliva on dental caries. J Am Dent Assoc 2008;139:11S-7S.
12. Orsi N. The antimicrobial activity of lactotransferrin: Current status and perspectives. Biometals 2004;17:189-96.
13. Gonzalez-Chavez SA, Arevalo-Gallegos S, Rascon-Cruz Q. Lactoferrin: Structure, function and applications. Int J Antimicrob Agents 2009;33:e301-8.
14. Liu D, Wang X, Zhang Z, Teng CT. An intronic alternative promoter of the human lactotransferrin gene is activated by Ets. Biochem Biophys Res Commun 2003;301:472-9.
15. Nariyama M, Shimizu K, Uematsu T, Maeda T. Identification of chromosomes associated with dental caries susceptibility using quantitative trait locus analysis in mice. Caries Res 2004;38:79-84.
16. Actor JK, Hwang SA, Kruzel ML. Lactoferrin as a natural immune modulator. Curr Pharm Des 2009;15:1956-73.
17. Vieira AR, Modesto A, Marazita ML. Caries: Review of human genetics research. Caries Res 2014;48:491-506.
18. Wang M, Qin M, Xia B. The association of Enamelin, Lactoferrin, and tumour necrosis factor alpha gene polymorphisms with high caries susceptibility in Chinese children under 4 years old. Arch Oral Biol 2017;80:75-81.
19. Kruzel ML, Zimecki M, Actor JK. Lactoferrin in a context of inflammation induced pathology. Front Immunol 2017;8:1438.
20. Sikorska MH, Mielnik-Blaszczak M, Kapec E. The relationship between the levels of SigA, lactotransferrin and alpha (1) proteinase inhibitor in saliva and permanent dentition caries in 15-year-olds. Oral Microbiol Immunol 2002;17:272-6.
21. Jentsch H, Beetke E, Gocke R. Salivary analyses and caries increment over 4 years an approach by cluster analysis. Clin Oral Investig 2004;8:156-60.
22. 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.
23. Salman HA, Senthilkumar R. Genotypic variations of mutans streptococci isolated from dental caries by REP-PCR. Baghdad Sci J 2020;17:1133.
24. Vieira AR, Marazita ML, Goldstein-McHenry T. Genome-wide scan finds suggestive caries loci. J Dent Res 2008;87:435-9.
25. World Health Organization. Oral Health Surveys Basic Methods 5th Edition, WHO Press, Avenue Appia/Geneva/Switzerland. 2013. www.who.int/oral_health.
26. Muhlemann HR. Oral epidemiology-caries. In: Zeitschriften-Verlag B, Quintessenze D. Introductionto oral preventive medicine; 1976. pp. 73-100.
27. Navazesh M, Kumar S. Measuring salivary flow: Challenges and opportunities. JADA 2008;139:35S-40S.
28. Alyousef YM, Borgio JF, AbdulAzeez S, Al-Masoud N, Al-Ali AA, Al-Shwaimi E, *et al*. Association of MBL2 gene polymorphism with dental caries in Saudi children. Caries Res 2017;51:12-6.
29. Doetzer AD, Brancher JA, Pecharki GD, Schlipf N, Werneck R, Mira MT, *et al*. Lactotransferrin gene polymorphism associated with caries experience. Caries Res 2015;49:370-7.