Association of a genetic variant (rs689466) of Cyclooxygenase-2 gene with chronic periodontitis in a sample of Iraqi population

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ABSTRACT

Background: periodontitis is a chronic inflammatory disease causing destruction of the tooth supporting structures, initiated by dental plaque and modified by environmental and genetic risk factors. Cyclooxygenase-2 (COX-2) enzyme is responsible for the production of prostaglandin E2, an important mediator in the chronic periodontitis (CP) pathogenesis. Polymorphisms in COX-2 gene have linked to CP in different populations.

Aim: To study the association between Cyclooxygenase-2 single nucleotide polymorphism rs689466 (-1195A/G SNP) and chronic periodontitis in a sample of Iraqi population.

Methods: One hundred Iraqi subjects divided into two groups: case group consisted of 70 CP patient (35 males and 35 females) with age range 30-55 years, and control group consisted of 30 racially matched healthy subjects (15 males and 15 females) with age range 30-50 years. Clinical periodontal parameters including plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) were recoded for all participants. 3ml of venous blood was collected from each participant for isolating genomic DNA. Genotyping of the rs689466 in COX-2 gene was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: The frequency of G allele carriers was significantly more prevalent in the case group compared to control group (P= 0.041), and allele G was associated with greater susceptibility for chronic periodontitis compared to allele A (OR=1.4).

Conclusion: COX-2 (rs689466) polymorphism may be associated with increased chronic periodontitis susceptibility.

Key words: chronic periodontitis, cyclooxygenase-2, rs689466 polymorphism. **(Received: 10/8/2018; Accepted: 4/9/2018)**

INTRODUCTION

Chronic periodontitis (CP) is a complex, multifactorial inflammatory disease of the tooth supporting tissues leading to gradual irreversible tissue destruction and may eventually lead to tooth loss [(1)]. The primary factor that initiates and maintains periodontal inflammation is the dental plaque biofilm that forms on teeth surfaces in the absence of oral hygiene [(2)].

Periodontitis develops from a pre-existing gingivitis which is reversible gingival inflammation. Individuals are not equally susceptible to periodontitis, in other words; not all cases of gingivitis progress to periodontitis in the presence of plaque deposits [(3)]. The inflammatory response is responsible for most of the tissue destruction in periodontitis. Therefore, the nature of the host inflammatory-immune response is the major determinant of host susceptibility [(4)]. Susceptible individuals in whom gingivitis rapidly progress to periodontitis have a hyper inflammatory response characterized by increased production of proinflammatory mediators and oxidative stress [(5-7)].

Many environmental and genetic factors influence the inflammatory response and the interaction of these factors determine whether the individual is susceptible to periodontitis or not [(8)].

Identification of the risk factors involved in the pathogenesis of periodontitis is important for better disease diagnosis and management [(9)].

Periodontitis has been shown to have an inherited basis of about 50%, meaning that genetic factors play an important role in disease susceptibility [(10)]. Genetic polymorphism in the molecules involved in periodontitis pathogenesis have been linked to increased risk for periodontitis in certain populations [(11, 12)].

Cyclooxygenase-2 (COX-2) enzyme is one of the most effective mediators in the pathogenesis of periodontitis via converting arachidonic acid to prostaglandins [(13)], it is the inducible form of the two cyclooxygenase isoforms (COX-1 and COX-2) [(14)]. Bacterial endotoxins and proinflammatory cytokines stimulate COX-2 expression which in turn increases the production of prostaglandin E2 (PGE2) in the periodontal tissues [(15)].

PGE2 causes more inflammation, tissue destruction and bone resorption. COX-2 expression is increased in periodontitis [(16)]. Polymorphisms (most commonly single nucleotide polymorphisms (SNPs)) in the COX-2

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gene may affect gene expression rate and thus periodontitis susceptibility [17].

SNPs in the COX-2 gene that have been linked to periodontitis susceptibility include rs689466, rs20417 and rs5275 [18]

In the present study we genotyped a sample of Iraqi population for rs689466 (–1195AG) SNP of COX-2 and tested the association of this SNP with chronic periodontitis.

MATERIALS AND METHODS

Study design and subjects:

This case-control study consisted of 100 Iraqi subjects of similar ethnic backgrounds recruited from the department of periodontics, college of Dentistry/University of Baghdad and the Iraqi national blood bank during the period from October 2017 to January 2018. Informed consent was obtained from each participant, and a questionnaire was used to record the background information, dental and medical histories of the participants.

Subjects were divided into two groups: control group (30 periodontally healthy subjects) and chronic periodontitis group (70 CP patients)

Criteria of the international classification of periodontal diseases and condition were used for diagnosing chronic periodontitis patients [19].

Ethical approval was obtained from the ethical committee of the college of Dentistry/University of Baghdad.

Clinical periodontal examination:

Periodontal status of all the participants was examined by the same examiner using UNC-15 periodontal probe and the following periodontal parameters were recorded: plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level.

Blood sample collection and DNA isolation:

3ml of the venous blood was collected in ethylene diamine tetra acetic acid (EDTA) tube using venipuncture method. DNA was isolated from the whole blood samples using gSYNC™ DNA extraction kit (Geneaid,Taiwan) and following the manufacturer's instructions. DNA samples were quantified by using a Nano-drop spectrophotometer then stored at –70°C.

Genotyping of COX-2 rs689466 polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Polymerase chain reaction (PCR):

PCR was performed using a ready to use mixture **(**AccuPower® PCR PreMix from Bioneer/ Korea) which is supplied in 0.2ml tubes, each tube contains Taq polymerase (1U), Deoxynucleoside triphosphates (dNTPs) each 250µM, 1.5mM MgCl2, Stabilizer and tracking dye. DNA sample and primers were added to the contents of the PCR PreMix tubes according to the manufacturer's instructions, then the tubes were transferred to a thermal cycler to complete the PCR according to the following program: initial denaturation at 95°C for 5 minutes, 30 PCR cycles consisting of denaturation at 95°C for 30 seconds, annealing at 58°C for 45 seconds and extension at 72°C for 45 seconds followed by final extension at 72°C for 5 minutes. The following primers were used: forward primer 5'ccctgagcactacccatgat3' and reverse primer 5'ccctgagcactacccatgat 3' [17].

The anticipated PCR product (273bp) was visualized using agarose gel electrophoresis on a 1.5% gel concentration captured under UV light (figure 1).

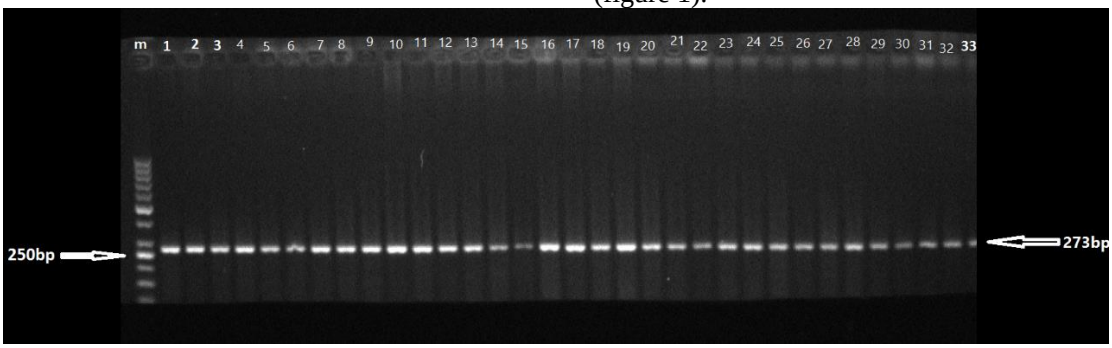

Figure 1: Results of the agarose gel electrophoresis of the PCR product for samples 1 to 33; m, DNA marker.

Restriction fragment length polymorphism (RFLP):

Pvull enzyme (SibEnzyme, Russia) was used for digesting the PCR product in order to produce fragments of different lengths to differentiate between different genotypes of rs689466 (-1195A/G SNP). In the presence of allele G at the position –1195, Pvull enzyme cuts the 273bp PCR product into two fragments (220bp + 53bp), while in the absence of allele A the enzyme couldn't cut, thus the 273bp fragment remains the same.

10 µl the PCR product of each sample was mixed with 1µl of the Pvull enzyme, 2µl of the SE Buffer, 1µl of the BSA and 1µl of the deionized distilled water. Then incubated in a thermal cycler at 37°C for 3 hours. The digestion product was separated by agarose gel electrophoresis using 3% gel concentration and visualized under UV light. The three genotypes were differentiated according to the band size. 273bp band indicated AA genotype, 220bp band indicated GG genotype and the presence of both 220bp and 273bp bands indicated AG genotype. 53bp was not visible. (Figure 2).

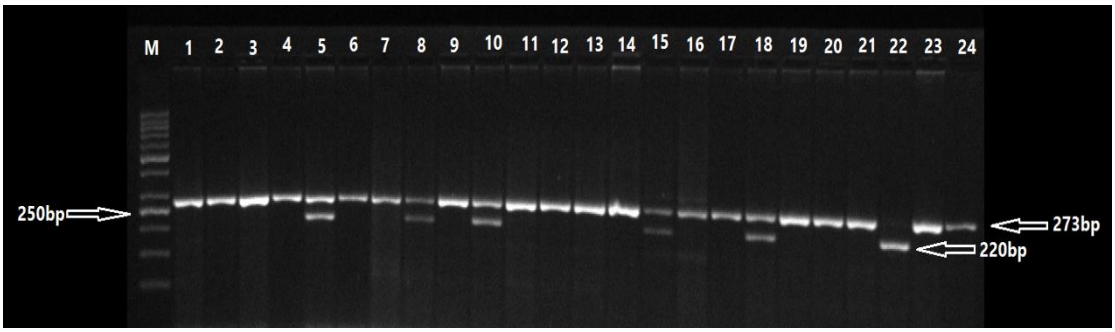

Figure 2: **Agarose gel electrophoresis of restriction digestion product for samples (1-24). M, DNA marker; 273bp, AA genotype; 220bp, GG genotype; 273+220, AG genotype.**

Statistical analysis

The statistical analysis system (SAS) program was used to perform statistical analysis. Descriptive statistics included: number, percentage, range, mean and standard deviation. t-test was used to compare between means and Chi-square to compare between percentages and genotype distributions. Results were considered significant when the probability P≤0.05. Odds ratio (OR) was used to express the association of genotypes with disease risk. OR=1 indicates no association, OR<1 indicates reduced risk and OR>1 indicates increased risk.

RESULTS

Characteristics of the study subjects (CP group and control group) are given in table 1. The mean age (±SD) for the control group was 42.03 (±5.70), and for CP group was 45.86 (±7.12). Male: female number was 15:15 in the control group and 35:35 in the CP cases.

Table 1: characteristics of study subjects

Characteristics of controls and CP cases			
		Control group(n=30)	**CP group(n=70)**
Age	Range	30-50	30-55
	Mean (±SD)	42.03 (±5.70)	45.86 (±7.12)
Gender	Male no (%)	15(50%)	35(50%)
	Female no (%)	15(50%)	35(50%)

Table 2 shows the clinical periodontal parameters. The difference in the PLI and GI was significant between cases and controls (p=0.00146 and 0.0016 for PLI and GI respectively). The percentage of bleeding sites (BOP score 1) in the CP group was 61.71%. The mean Probing pocket depth (PPD) (±SD) in the CP group was 3.094(±0.642), and the mean clinical attachment level (CAL) (±SD) was 4.33(±0.913).

Table 2: Clinical periodontal parameters

	Control group (n=30)	CP group (n=70)	P-value
Mean PLI (±SD)	0.827 (±0.228)	1.979 (±0.245)	0.00146
Mean GI (±SD)	0.631(±0.158)	1.632 (±0.298)	0.0016
BOP score 1 (%)	---	61.71%	---
Mean PPD (±SD)	---	3.094mm (±0.642)	---
Mean CAL (±SD)	---	4.33mm (±0.913)	---

Table 3 shows COX-2 rs689466 genotypes and alleles and their prevalence in the study subjects. 74% of the people were AA homozygous, 23% were AG heterozygous and 3% GG homozygous. The dominant model (combining AG+GG in one group called allele G carriers) was used.

Table 3: Distribution of COX-2 rs689466 genotypes and alleles in the study population.

Genotypes	Frequency in the study population	Total
AA homozygous	74 (74%)	n=100
AG heterozygous	23 (23%)	
GG homozygous	3 (3%)	
Allele G carriers (AG+GG)	26 (26%)	
Alleles		
A (Wild)	171 (85.5%)	2n=200
G (Mutant)	29 (14.5%)	

Distribution of the COX-2 rs689466 genotypes and alleles in CP cases and controls is illustrated in table 4. AA homozygous was significantly higher in the control group compared to CP group (P=0.047). The dominant model (AG+GG) revealed that allele G carriers were significantly more prevalent in the CP cases compared to controls (0.041), and possess greater risk for disease as indicated by elevated odd ratio (1.6).

Table 4: Distribution of COX-2 rs689466 genotypes and allele frequency between controls and chronic periodontitis cases.

COX-2 rs689466		**Controls**	**CP cases**	**OR**	**p-value**
Genotypes		**n=30**	**n=70**		
AA	No (%)	24 (80%)	50 (71.43%)	0.625	0.047
(AG+GG)	No (%)	6 (20%)	20 (28.57%)	1.6	0.041
Allele frequency		**2n=60**	**2n=140**		
A	No (%)	53 (88.33%)	118 (84.29%)	0.708	0.093
G	No (%)	7 (11.67%)	22 (15.71%)	1.411	0.1447

Table 5 shows the distribution of the COX-2 rs689466 genotypes and alleles between males and females. The prevalence of AA genotype was equal for both males and females (74%, P=1.00: OR=1). The prevalence of the mutant allele carriers (AG+GG) was also equal (26%) in both genders with a non-significant difference in allele frequency between males and females (allele A frequency was 85% in males and 86% in females, p=0.887: OR=0.92. While allele G frequency was 15% in males and 14% in females, p=0.887: OR=1.08).

Table 5: Distribution of COX-2 rs689466 genotypes and allele frequency between males and females.

COX-2 rs689466		**Males**	**Females**	**OR**	**p-value**
Genotypes		**n=50**	**n=50**		
AA	No (%)	37 (74%)	37 (74%)	1	1.00
(AG+GG)	No (%)	13 (26%)	13 (26%)	1	1.00
Allele frequency		**2n=100**	**2n=100**		
A	No (%)	85 (85%)	86 (86%)	0.92	0.887
G	No (%)	15 (15%)	14 (14%)	1.08	0.887

DISCUSSION

Periodontitis is a complex disease, although microbial dental plaque is the initiating factor, other environmental and genetic risk factors play important role in the pathogenesis. Identification of these risk factors is important for effective disease prevention and management[2]. As shown in (Table 2), the significant difference in the PLI and GI levels between controls and cases is explained by the fact that dental plaque is the primary cause of periodontal disease [2, 11, 20].

In the pathogenesis of periodontitis, the induction of COX-2 by bacterial endotoxins and pro-inflammatory cytokines results in the production of increased amounts of prostaglandin E2 (PGE2) in periodontal tissues [15]. PGE2 is a potent inflammatory mediator and a stimulator of osteoclastogenesis and bone resorption [21, 22].

Also it stimulates the production of matrix metalloproteinases causing further tissue destruction. It has been shown that the concentration of PGE2 in the gingival crevicular fluid is correlated with periodontal disease severity and reduced after treatment. COX-2 is over expressed in periodontitis [16, 23], while the inhibition of COX-2 reduces the rate of bone resorption and periodontal disease progression [24]. Variations in the COX-2 gene have been linked to many inflammatory and proliferative diseases. COX-2 gene polymorphisms may influence periodontitis susceptibility by influencing the host's inflammatory response. The most common type of genetic polymorphisms are the single nucleotide polymorphisms (SNPs) in which a single nucleotide at a specific point is substituted be another nucleotide [25]. Three SNPs rs689466, rs20417 and rs5275 in the COX-2 gene have been reported to be associated with periodontitis risk in different populations. However, the results were inconsistent [12]. In the present study the COX-2 rs689466 was investigated in a sample of Iraqi population and tested for association with chronic periodontitis. This SNP has been previously investigated in Chinese, European, and North Indian populations [18, 26-28]. The results of our study showed that the distribution of the SNP genotypes in the Iraqi population (Table 3) is different from genotype distributions of the same SNP in the previously mentioned populations. AA homozygous was the most prevalent genotype in both controls and cases. However, it was significantly more prevalent in controls compared to CP cases and associated with reduced disease risk, while allele G carriers were significantly more prevalent in CP group compared to controls and associated with higher disease risk as indicated by elevated odd ratio (Table 4). A comparison between males and females showed a nearly equal distribution of COX-2 rs689466 genotypes and alleles between males and females (Table 5) suggesting that gender specific genetic effect may not contribute to the overall periodontitis risk, this agrees with Michalowicz et al [10].

As a conclusion, this study revealed that the genetic variant (rs689466) in the promotor area of COX-2 gene may be associated with CP susceptibility in Iraqi population. However, the study should be replicated in a larger independent sample of similar racial background.

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الخلاصة

الخلفية العلمية: التهاب دواعم السن هو مرض التهابي مزمن يصيب الانسجة الداعمة للسن مسببا ضررا دائما فيها. الالتهاب ينشأ بشكل أساسي من تراكم الصفيحة الجرثومية على سطح الاسنان ويخضع لتأثير العوامل البيئية والوراثية. انزيم السايكلواوكسيجينيز2 مسؤول عن انتاج بروستاجلاندين E2 الذي يقوم بدورهام في إمراضية التهاب دواعم السن. تعدد اشكال النيوكليوتيدة لجين السايكلواوكسيجينيز2 يرتبط بزيادة خطر المرض في مجاميع عرقية معينة.
أهداف الدراسة: أجريت هذه الدراسة للتحقق من وجود علاقة بين تعدد اشكال النيوكليوتيدة في الموقع(rs689466) لجين السايكلواوكسيجينيز 2 ومرض التهاب دواعم السن المزمن في عينة من المجتمع العراقي.
المواد وطرق العمل: شملت هذه الدراسة على 100 عراقي تم تقسيمهم إلى مجموعتين: مجموعة الحالات تكونت من 70 مريض (35 من الذكور و35 من الاناث) مصابين بالتهاب دواعم السن ومجموعة السيطرة تكونت من 30 شخص (15 من الذكور و15 من الاناث) بمعدل اعمارمن 30-50 سنة, لا يشكون من التهاب دواعم السن. مؤشرات ما حول الاسنان التي تم تسجيلها للمشاركين شملت: مؤشر الصفيحة الجرثومية, مؤشر التهاب اللثة, مؤشر النزف عند التسبير, عمق الجيوب اللثوية و مستوى الارتباط السريري. تم جمع 3 مل من الدم الوريدي من كل مشترك. تم استخلاص الحمض النووي(الدنا) من عينات الدم لغرض التنميط الجيني. تم إجراء التنميط الجيني لتعدد أشكال النيوكليوتيدة في الموقع(rs689466) لجين السايكلواوكسيجينيز2 باستخدام طريقة:
Polymerase chain reaction-restriction fragment length polymorphismوالترحيل الكهربائي باستخدام جل الاكاروز.
النتائج: أظهرت النتائج أن تردد الحاملين لأليل G كان مرتفعا معنويا في حالات التهاب دواعم السن المزمن مقارنة بمجموعة السيطرة (P=0.041) كما ان أليل G مرتبط بزيادة احتمالية الاصابة بالتهاب دواعم السن مقارنة بالأليل A (OR=1.4) .
الاستنتاج: تعدد أشكال النيوكليوتيدة في الموقع(rs689466) لجين السايكلواوكسيجينيز2 قد يرتبط بزيادة القابلية للإصابة بالتهاب دواعم السن المزمن.