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Research Paper

Tumor Necrosis Factor-Alpha – 308 Gene Polymorphism in the Association between Gestational Diabetes Mellitus and Chronic Periodontitis in South Indian Population

Dhayanand John Victor, Sangeetha Subramanian, Prakash Ponnudurai Samuel Gnana, Bob Jacob Joseph[1]

Department of Periodontics, SRM Dental College, Chennai, Tamil Nadu, India, [1]Kedar Dental Centre, Sharjah, UAE

Abstract

Objective: To investigate if tumor necrosis factor-alpha (TNF-α) G308A polymorphism influences the association between generalized chronic periodontitis (GCP) and gestational diabetes mellitus (GDM) in South Indian population. **Materials and Methods:** Clinical parameters were recorded in 99 CP patients who were categorized into two groups. The control group consisted of 49 individuals without GDM. The test group included fifty GCP patients with GDM. Genomic deoxyribonucleic acid was extracted from all the participants. The locus − 308 of TNF-α has frequently been associated with CP and DM, and hence this locus has been chosen for the study. Genotyping was carried out using allele-specific-polymerase chain reaction, and the frequencies of genotype were analyzed between the groups. **Results:** The distribution of genotype and allele frequencies showed significant differences between the study groups. The frequency of GA genotype was significantly higher in CP patients with GDM compared to patients without GDM ($P = 0.014$). The frequency of A allele was also significantly higher in GDM patients (31%) than non-GDM patients (13%) with CP. **Conclusion:** TNF-α G308A polymorphism could be a risk factor for the association between GDM and CP in South Indian population.

Keywords: Chronic periodontitis, gene polymorphism, gestational diabetes mellitus, tumor necrosis factor-alpha polymorphism, tumor necrosis factor-α

Introduction

Periodontal disease is an infectious-inflammatory process that results in the destruction of periodontal tissue including alveolar bone supporting the teeth. Several risk factors such as microbial, immunological, systemic, environmental, and genetic factors play a vital role in the progression of the disease.[1] An increased association of periodontal disease with gestational diabetes mellitus (GDM) has been observed in various studies.[2,3] Increased levels of tumor necrosis factor-alpha (TNF-α) and interleukin-6 among women with GDM suggest a role for infection and inflammation in its etiology.[4-6]

TNF is a pro-inflammatory cytokine which is considered to be one of the contributing factors for insulin resistance in diabetes.[7] TNF-α produced due to periodontal inflammation may further influence insulin sensitivity and may cause the manifestation of GDM. The *TNFA* gene is located on chromosome 6p21.3 within the major histocompatibility complex gene cluster. One of the best-analyzed polymorphisms is the G-to-A transition at position 308 within the promoter region.[7] The rare allele 2 (A308) has been linked with higher transcriptional activity[8] and an elevated TNF secretion.[9]

Several case–control studies have reported that genetic polymorphisms in the *TNF-α* gene is a putative risk factor for periodontitis. We hypothesized that single-nucleotide polymorphism of TNF-α (G308A) might contribute to increased strength of association between GDM and chronic periodontitis (CP). Therefore, the goal of this study was to

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Address for correspondence: Sangeetha Subramanian, 6A, 11th Street, New Colony, Adambakkam, Chennai - 600 088, Tamil Nadu, India.
E-mail: sangeetha_doc@yahoo.com

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evaluate TNF-α gene polymorphism at loci −308, in GDM patients with CP in South Indian population.

Materials and Methods

The 99 participants who had taken part in the present study were chosen from patients who attended the Department of Gynecology and Obstetrics at Government General Hospital, Madras Medical College, Chennai, Tamil Nadu, India. The patients included in this study were ethnic Tamil-speaking South Indians. All the patients were of aged 20–30 years and in the 2nd trimester of pregnancy.

Each patient was given a detailed verbal description of the purpose and nature of the study including clinical measurements and sample collection. The study was approved by the Institutional Scientific Committee and Ethical Review Board of SRM Dental College and Madras Medical College, Chennai. The Ethical Committee's permission letter number is SRMU/M and HS/SRMDC/2010–2013/MDS-PG Student/502. The study groups consisted of 99 CP patients comprised of 50 GDM patients in the test group (Group A) and 49 non-GDM women in the control group (Group B).

Criteria for selection

CP patients presented with clinical attachment loss ≥3 mm and probing pocket depth (PPD) ≥5 mm involving at least two sites were selected for the study. Patients diagnosed with GDM in the first or second trimester were included as the study group and systemically healthy pregnant women were included as controls. Exclusion criteria applied for both groups included presence of other local modifiers of periodontal disease such as food impaction, overhanging margins and malocclusion, smokers, and patients who received periodontal treatment 6 months prior to the study.

Criteria for assessment of gestational diabetes

GDM was assessed based on the laboratory reports obtained from the hospital. Patients were given 100 g of oral glucose and their blood glucose levels were evaluated at baseline (fasting) and at 1, 2, and 3 h.

The diagnosis of GDM was made if one or more of the following criteria were met: fasting glucose ≥5.1 mmol/L (92 mg/dL), 1-h glucose ≥10 mmol/L (180 mg/dL), 2-h glucose ≥8.5 mmol/L (153 mg/dL), or 3-h glucose ≥7.75 mmol/L (140 mg/dL).

Clinical evaluation of the participants

Patients' medical, dental, and clinical parameters were recorded. A complete periodontal examination was carried out which included PPD and clinical attachment level measured at six sites around each tooth using a University of North Caroline probe. The indices taken for the study were Oral Hygiene Index-Simplified, Plaque Index, and Modified Sulcular Bleeding Index.

Sample collection and DNA extraction

From each patient, 2 ml of venous blood was collected by venepuncture and was transferred into a ethylenediaminetetraacetic acid-coated vacutainter and stored at −20°C. Genomic DNA was isolated from peripheral leukocytes using the QIAamp DNA Blood Mini Kit, Qiagen, following manufacturer's instructions. A total of 99 DNA samples were genotyped for G308A using allele-specific polymerase chain reaction (PCR). Primers used for amplifying the G308A gene were as follows: Forward primer: 5'AGGCAATAGGTTTTGAGGGCCAT3' and Reverse primer: 5'TCCTCCCTGCTCCGATTCCG 3'.

The PCR reaction was performed at a final volume of 20 µl containing 2 µl template DNA, 10 µl of Master Mix, 0.3 µl each of forward and reverse primer, and 0.5 µl of *Taq* DNA polymerase. The reaction mixture was subjected to initial denaturation at 94°C for 5 min followed by 20–35 cycles performed at denaturation 94°C for 5 min, annealing 57°C for 45 s, and extension 72°C for 1 min. The final extension was done at 72°C for 5 min. PCR products were analyzed in 2% agarose gel electrophoresis and were visualized with ethidium bromide staining under ultraviolet light. Hundred base pairs' ladder was used as the standard molecular weight marker.

Statistical analysis

Statistical analysis was done with SPSS software developed by IBM Corporation Version 17.0. Normal distribution, mean and standard deviation were calculated for all clinical parameters assessed for this study. Mean values were compared between the two groups using the *t*-test. The Chi-squared test was applied to examine the differences in genotype distribution and allele frequency between test and control groups. Odds ratio (OR) and its confidence interval (CI) with *P* value were used to describe the strength of association. In the present study, $P < 0.05$ was considered as the level of statistical significance.

Results

A total of 99 South Indians who participated in the study were genotyped for TNF-α gene polymorphism. The clinical presentation and demographic characteristics of the patients are summarized in Table 1. There were no statistically significant differences found when the clinical parameters were compared between the groups.

Distribution of genotypes and allele frequency among the study groups

Distribution of homozygous GG genotype was higher (75.51%) in non-GDM individuals, while the heterozygous GA genotype occurred in 42% of GDM patients. Pearson's $\chi^2 = 8.553$ ($P = 0.014$) and $\chi^2 = 8.01$ ($P = 0.005$) were obtained when either the three genotypes or alleles were compared among the test and control groups [Table 2]. Data analysis showed higher distribution of G allele in non-GDM with CP ($n = 85$, 86.73%) and the A allele was found to be higher in GDM patients with CP ($n = 31$, 31%). The number of participants with GA genotypes was higher in gestational diabetics with CP category compared to non-GDM category with OR of 2.93 and 95% CI of 1.20–7.18 with statistical significance set at $P = 0.018$ [Table 3].

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Table 1: Clinical and demographic characteristics

Variables	Mean±SD		*P*
	GDM with GCP (*n*=50)	Non-GDM with GCP (*n*=49)	
Age	22.25±6.92	24.57±6.84	
Oral Hygiene Index-S	2.22±0.21	1.91±0.19	0.09
Plaque Index	2.41±0.21	2.13±0.11	0.113
mSBI	1.71±0.29	1.1±0.22	0.271
PPD	6.28±0.51	5.95±0.92	0.301
CAL	5.58±0.66	5.27±0.82	0.237

mSBI=Modified Sulcular Bleeding Index, PPD=Probing pocket depth, CAL=Clinical attachment level, SD=Standard deviation, GDM=Gestational diabetes mellitus, GCP=Generalized chronic periodontitis

Table 2: Distribution of tumor necrosis factor-alpha genotypes and allele frequency between test and control groups

	GDM with GCP (*n*=50), *n* (%)	Non-GDM with GCP (*n*=49), *n* (%)
Genotypes		
GG	24 (48)	37 (75.51)
GA	21 (42)	11 (22.45)
AA	5 (10)	1 (2.04)
c, *P*	8.553, 0.014	
Allele		
G	69 (44.8)	85 (55.19)
A	31 (70.45)	13 (29.54)
Pearson's χ^2 analysis, *P*	8.01, 0.005	

Pearson's χ^2 analysis was used to analyze the distribution of genotypes and allele frequency between the study groups. GDM=Gestational diabetes mellitus, GCP=Generalized chronic periodontitis

Table 3: Distribution of genotypes and odds ratio with 95% confidence interval

Genotype	GDM	Non-GDM	OR	*P*
GG	24	37	1.00	
GA	21	11	2.93 (1.20-7.18)	0.018*
AA	5	1	7.70 (0.84-70.14)	0.070

Logistic regression analysis was performed to examine the distribution of genotypes with OR and CI. *$P \leq 0.05$. CI=Confidence interval, OR=Odds ratio, GDM=Gestational diabetes mellitus, GCP=Generalized chronic periodontitis

Discussion

In our attempts to understand periodontal disease, research has highlighted on the links between periodontal and systemic disease. Among the most significant associations highlighted, clinical research has constantly emphasized the two-way link between periodontal disease and DM.[10] It has been well documented that periodontal disease is more prevalent in individuals with type 2 diabetes compared to healthy controls. Periodontitis has been referred to as the sixth complication of DM, and this has been attributed to the consequences of a long duration of elevated blood glucose levels. A significant association was demonstrated between periodontal diseases and GDM.[2] A study by Novak *et al*. in 2006[11] stated that women with GDM were at greater risk for developing more severe periodontal disease than pregnant women without GDM.

There was a significant elevation of TNF-α and IL-1β at sites with periodontal attachment loss in patients with GDM.[12] This increased titer of pro-inflammatory cytokines in patients with periodontal disease and GDM can be explained either by the fact that the diabetic state caused a hyperinflammatory condition or by an inherent difference of the genetic makeup of an individual, causing these patients who are gestational diabetics to produce exaggerated levels of TNF-α at sites of inflammatory stimuli. An increased association between periodontitis and type 2 diabetes mellitus was observed in patients with TNF-α gene polymorphism at −308 loci.[13] Therefore, the goal of this study was to evaluate the association of TNF-α gene polymorphism at loci −308, in GDM patients with CP. The Indian population is recognized to be genetically diverse, and hence to avoid confounding effects that may arise due to genetic diversity, the study was conducted on a racially defined, Tamil-speaking individuals from South India.[14]

In the present study, the frequency of the G/A genotype was significantly higher in the CP patients with GDM when compared to patients without GDM. The G308A allele of the TNF-α gene has been found to increase TNF-transcription[8] and secretion.[15] TNF-α has been shown to influence glucose metabolism, antagonizing insulin action by preventing insulin-stimulated tyrosine phosphorylation at the insulin receptor.[16] TNF-α also enhances serine phosphorylation of insulin receptor substrate (IRS)-1. These actions of TNF-α confer a state of insulin resistance. In pregnancy, it is suggested that insulin receptor and IRS-1 tyrosine phosphorylation are compromised, and serine phosphorylation is increased in late gestation in skeletal muscle.[17] Therefore, it seems plausible that elevated levels of TNF-α in late gestation could attenuate insulin signaling, thus causing the decreased insulin sensitivity observed in pregnancy.

The results suggest that patients presenting with a G > A polymorphism at the − 308 loci of the TNFA gene have almost a threefold increased odds of developing GDM and CP due to an increased production of the pro-inflammatory cytokine TNF-α. This study also suggests that the association seen between GDM and CP is based on a heritable genetic polymorphism and not due to the hyperglycemic effects of GDM on the periodontium. This would further imply that patients developing early CP may be at risk for future GDM/type 2 DM due to the genetic polymorphism.

These results seem to substantiate the results of the NHANES I study which found that the baseline periodontal disease was an independent predictor of DM in an over two-decade follow-up. Their results suggested that patients with a baseline periodontal disease had a twofold increased odds of

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developing DM.[18] The results of the current study found that patients with periodontal disease and GDM had a threefold increased odds of having a G > A polymorphism at the −308 loci of the TNF-α gene.

It is then plausible that the increased incidence of type 2 diabetes, in patients who had baseline periodontitis, was due to the TNF-α gene polymorphism that primed these individuals to develop CP earlier in life and to become more susceptible to develop DM at a future date. If these findings are established, patients who develop early periodontal disease and who present with a TNF-α gene polymorphism may then receive targeted preventive medical treatment to ensure that these individuals do not acquire environmental factors that will result in the clinical presentation of DM.

While this study had patients in two groups who were matched, but for the presence of GDM, various other studies have shown a significantly higher incidence of periodontitis among gestational diabetics.[2,11] Further studies will need to be carried out in differing populations and also across the spectrum of periodontal health to disease to have a more clearer picture on the role of genetic polymorphism at this site.

Conclusion

This is the first study that has researched the role of TNF-α genetic polymorphism in the association between GDM and CP. The results of this study offer the probability of hope for sound understanding of the link between these diseases as well as the probability of offering appropriate preventive measures for periodontal disease as well as DM. From the results of the study, it was observed that TNF-α G308A gene polymorphism could be a risk factor for the association of GDM with CP in South Indian population. However, larger studies are required across varied ethnic backgrounds and periodontal health status before finding the association of genetic polymorphism at the − 308 loci of the TNF-α gene, in GDM and CP, which is confirmed to be a risk factor for these diseases.

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Conflicts of interest

There are no conflicts of interest.

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