

EVALUATION OF LEVEL OF TNF- α IN CHRONIC PERIODONTITIS PATIENTS WITH GESTATIONAL DIABETES MELLITUS AFTER PHASE I PERIODONTAL THERAPY

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ABSTRACT

Background: There is little number of studies that address the inter-relationship of gestational diabetes mellitus (GDM) and periodontitis during pregnancy. Hence, this study was conducted to provide further evidence in the possible association between chronic periodontitis and GDM by evaluating the level of **Tumor necrosis factor-alpha (TNF- α)** of chronic periodontitis patients with GDM after phase I periodontal therapy.

Subjects and methods: This study was conducted on 40 subjects divided into 2 groups: 20 pregnant females suffering from gestational diabetes mellitus associated with moderate to severe chronic periodontitis and 20 systemically free pregnant females suffering from moderate to severe chronic periodontitis. The periodontal status of the subjects was assessed at baseline before phase I periodontal therapy and 2 months after completion of the treatment: All subjects have been screened by comprehensive periodontal examination and full periodontal charts were obtained. The following clinical parameters were assessed to determine the clinical periodontal status of patients: Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL). Gingival crevicular fluid (GCF) and serum samples were collected from both study groups to detect TNF- α levels. Regarding the assessment of TNF- α , Real-time reverse-transcriptase Polymerase Chain Reaction (RT) PCR technique was used.

Results: Results of the present study observed statistically significant reduction in TNF- α levels after 2 months from phase I periodontal therapy. The current study showed that, there was a statistically significant positive (direct) correlation between TNF- α level, PI, GI, PPD and CAL measurement at baseline and after 2 months from phase I periodontal therapy.

Conclusion: It is shown that the levels of TNF- α in GCF and serum before treatment were higher in GDM group (group 1) than in the control group. Thus, the examination of TNF- α may enhance the understanding of pathogenesis of periodontitis and GDM and its assessment in the treatment process may result in better control of the disease.

KEYWORDS: Chronic periodontitis, GDM, TNF- α .

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INTRODUCTION

Periodontal diseases are a group of oral inflammatory diseases that are influenced by host response factors. In the last decades, there was a consensus toward the converse relationship between periodontal disease and systemic disease¹⁻³. Evidence suggest that periodontal disease is an independent risk-factor for a number of significant systemic diseases so much so that they have brought a shift in the rationale about causality and the directionality of oral and systemic associations. This shift is encapsulated by the new term periodontal medicine^{4,5}.

Cytokines (Greek “cyto-”, cell; and “-kinos”, movement) are a family of signaling molecules that mediate and regulate immunity, inflammation, hematopoiesis, and many other cellular processes. TNF- α is considered as one of the adipokines which are group of over 600 bioactive molecules produced by adipose tissue that acts as paracrine and endocrine hormones^{6,7}.

When adipose tissue inflammation and dysfunction are established, adipokine secretion is significantly changed toward a diabetogenic, pro-inflammatory and atherogenic pattern. TNF- α is a pro-inflammatory cytokine that plays a central role in inflammatory reaction, alveolar bone resorption, and the loss of connective tissue attachment. It is positively correlated with matrix metalloproteinases (MMPs), Prostaglandin E2 (PGE2) and nuclear factor kappa ligand (RANKL) expression⁸⁻¹⁰.

Gingival crevicular fluid (GCF) is an inflammatory exudate that seeps into gingival crevices or periodontal pockets around teeth with inflamed gingiva. It can be collected from healthy gingival sulcus, although only in small amount. GCF represents the transudate of gingival tissue interstitial fluid produced by an osmotic gradient in the healthy periodontium. It contains different substances including: immunoglobulin, microorganisms, toxins, cells, and lysosomal enzymes and markers. GCF is regarded as a window

for non-invasive analysis of periodontal conditions, including markers of connective tissue and bone destruction¹¹⁻¹⁴.

Evaluation of the molecular markers of tissue destruction in serum was sought to clarify the possible interactions between periodontitis and various systemic diseases and conditions such as gestational diabetes mellitus GDM and adverse pregnancy outcomes). Serum provides information about the inflammatory stimulus and the response generated in circulation towards the periodontal pathogens that colonize the sub-gingival area^{15,16}.

The present study was conducted to evaluate the levels of TNF- α in GCF and serum in chronic periodontitis patients with or without GDM before and after phase I (non-surgical) periodontal therapy and its correlation to periodontal clinical parameters.

AIMS OF THE STUDY

The aim of the present study was to evaluate the possible effect of phase I (non-surgical) periodontal therapy on TNF- α in chronic periodontitis patient with GDM.

SUBJECTS, MATERIALS AND METHODS

Study Population:

Forty adult pregnant female subjects selected from the obstetrics & gynecology department, Faculty of Medicine, Cairo University & the obstetrics & gynecology department, Faculty of Medicine, Misr University for Science & Technology and El Galaa Maternity Hospital. Participants were pregnant females aged between 20-40 years, at the second trimester of pregnancy, not taking any local or systemic medications & had no periodontal therapy for the previous six months and were not using any mouth wash. Exclusion criteria were: Already diabetic participants, over 40 years of age, smokers, participants having known systemic disease other than cases of GDM group (group 1) and participants who had periodontal therapy at the last 6 months.

Cases and Controls:

The present study was conducted on 40 subjects divided into 2 groups of 20 subjects each: Group (1); Included 20 pregnant females suffering from GDM associated with moderate to severe chronic periodontitis. Group (2); Included 20 systemically free pregnant females suffering from moderate to severe chronic periodontitis acting as control group. All pregnant females included in this study had a moderate to severe chronic periodontitis and had been free from any systemic disease according to Cornell medical index except GDM cases in GDM group (group 1). All pregnant women have undergone a laboratory screening test for GDM consisting of fasting blood glucose test (no caloric intake for 8 hours) and 2 hours post-prandial glucose test¹⁷.

Assessment of periodontal condition:

All participants were screened by comprehensive periodontal examination and full periodontal charts were obtained. The following clinical parameters were assessed to determine the clinical periodontal status of patients: PI, GI, PD, and CAL¹⁸⁻²⁰.

The study design:

The periodontal status of the participants was assessed at baseline before phase I periodontal therapy and 2 months after completion of the treatment. All participants received phase I periodontal therapy that includes oral hygiene instructions, scaling and root planning under local anesthesia using sharp scalers, Gracey curettes and ultrasonic debridement.

Samples collection:

GCF samples were collected from the selected sites for assessment of TNF- α levels at baseline before initial periodontal therapy and 2 months after the completion of Phase I therapy. The GCF samples were collected using filter paper strips (2mm x 8mm). 5 ml of blood was collected from the antecubital fossa by venipuncture using

20gauge needle and 2ml syringes and immediately transferred to the laboratory. Samples were allowed to clot for 1 hour at room temperature centrifuged for 10 minutes (4°C) and serum was extracted. Collected GCF and serum samples were stored at -40°C before used for assay procedure.

Assessment of TNF- α in GCF and serum by PCR

Serum and GCF samples were assayed for TNF- α levels by PCR using Qiagen extraction kit (Qiagen, Valencia, CA, and USA) Serum and GCF samples were taken before the phase I periodontal therapy and 2 months after the completion of active therapy (debridement).

Statistical Analysis:

Values of clinical parameters and TNF- α levels were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data), so parametric tests were used for the comparisons.

RESULTS**Scores of TNF- α (GCF) and significance of the difference before and after treatment (paired t test) and between groups (unpaired t test):**

A higher mean score was recorded in GDM group (group 1). Unpaired t test revealed that this difference was statistically significant preoperatively ($p < 0.0001$), but insignificant post operatively ($P = 0.0534$). Treatment decreased the TNF- α (GCF) level in each group. Paired t test revealed that this decrease was statistically significant in GDM group (group 1) ($P < 0.0001$), but insignificant in control group ($p = 0.3200$). Comparing the percent decrease in both groups, unpaired t test revealed a greater significant decrease in GDM group (group 1) ($p < 0.0001$) as shown in table 1.

Scores of TNF- α (serum) and significance of the difference before and after treatment (paired t test) and between groups (unpaired t test):

A higher mean score was recorded in GDM group (group 1). Unpaired t test revealed that this difference was statistically significant preoperatively ($p < 0.0001$) and post operatively ($P = 0.0184$). Treatment decreased the TNF- α

(serum) level in GDM group (group 1) only. Paired t test revealed that this decrease was statistically significant in GDM group (group 1) ($P < 0.0001$), while the increase in control group was insignificant ($p = 0.2759$). Comparing the percent change in both groups, unpaired t test revealed a greater significant decrease in GDM group (group 1) ($p < 0.0001$) as shown in table 2.

TABLE (1) Scores of TNF- α (GCF) and significance of the difference before and after treatment (paired t test) and between groups (unpaired t test)

		Score			
		Group 1	Control	t value (unpaired t test)	P value (unpaired t test)
TNF- α (GCF) before	Mean	2.80	1.29	13.08	<0.0001*
	SD	0.42	0.30		
	Min	2.13	0.96		
	Max	3.43	1.67		
TNF- α (GCF)-post therapy	Mean	1.41	1.17	1.994	0.0534 ^{ns}
	SD	0.31	0.44		
	Min	0.96	0.82		
	Max	1.80	1.73		
T value (paired t test)		11.90	1.0077	-----	
P value (paired t test)		<0.0001*	0.3200 ^{ns}		

*significant ns=non-significant

TABLE (2) Scores of TNF- α (serum) and significance of the difference before and after treatment (paired t test) and between groups (unpaired t test)

		Score			
		Group 1	Control	t value (unpaired t test)	P value (unpaired t test)
TNF- α (Serum)-before	Mean	1.41	0.65	8.50	<0.0001*
	SD	0.34	0.21		
	Min	0.96	0.42		
	Max	1.88	0.99		
TNF- α (Serum)-post therapy	Mean	0.85	0.72	2.4634	0.0184*
	SD	0.14	0.19		
	Min	0.66	0.46		
	Max	0.99	0.91		
T value (paired t test)		0.681	1.1054	-----	
P value (paired t test)		<0.0001*	0.2759 ^{ns}		

*significant ns=non-significant at $p > 0.05$

TABLE (3) Correlation between TNF- α and Plaque index (Pearson correlation test)

Correlation of TNF- α (GCF)	R	R ²	Significance
1- PI (group1)	0.9117	0.8312	Strong positive
2- PI (control)	0.3509	0.1231	Weak positive

TABLE (4) Correlation between TNF- α and gingival index (Pearson correlation test)

Correlation of TNF- α (GCF)	R	R ²	Significance
1- PI (group1)	0.8427	0.7101	Strong positive
2- PI (control)	0.313	0.0908	Weak positive

TABLE (5) Correlation between TNF- α and PPD (Pearson correlation test)

Correlation of TNF- α (GCF)	R	R ²	Significance
1- PI (group1)	0.7449	0.5549	Moderate positive
2- PI (control)	-0.0246	0.0006	Weak negative

TABLE (6) Correlation between TNF- α and CAL (Pearson correlation test)

Correlation of TNF- α (GCF)	R	R ²	Significance
1- PI (group1)	0.8033	0.6453	Strong positive
2- PI (control)	0.0581	0.0034	Weak positive

DISCUSSION

Studies have shown that there are elevated inflammatory cytokines in patients with GDM and diabetes. Diabetes and periodontal inflammation

clearly interact, with overt diabetes associated with an increased risk of more severe periodontitis while periodontitis has been associated with worsened glycemic control in subjects with diabetes²¹⁻³⁰. Consequently, the present study was conducted to evaluate the levels of TNF- α in GCF and serum in chronic periodontitis patients with or without gestational diabetes mellitus GDM before & after phase I (non-surgical) periodontal therapy and its correlation to periodontal clinical parameters³¹.

Participants over 40 years of age were excluded from this study due to the effect of estrogen level decrease upon TNF- α . Moreover, smokers were excluded from this study as it was found that tissues exposed to tobacco carcinogens respond by expressing elevated levels of cytokines in those tissues presumably as a part of injury response mechanism³²⁻³⁵.

GCF provides an accurate representation of tissue and serum concentrations of inflammatory mediators. Evaluation of the molecular markers of tissue destruction in serum was sought to clarify the possible interactions between periodontitis and various systemic diseases and conditions such as GDM and adverse pregnancy outcomes³⁶⁻³⁹.

The suitability of TNF- α in GCF as a possible indicator of periodontal disease was first assessed by *Rossomando*⁴⁰. TNF- α is a pro-inflammatory cytokine that is often over expressed in periodontitis and is responsible for alveolar bone resorption during periodontitis. Moreover, TNF- α has been reported to be an insulin antagonist^{41,42}.

The evidence that insulin resistance is linked to TNF- α is well established. Additionally, TNF- α has been demonstrated to be the most significant predictor of pregnancy-induced insulin resistance and be more highly synthesized and released from the placenta compared with IL-6 or IL-8^{43, 44}. Therefore, TNF- α was the inflammatory marker of choice to be used to evaluate the inflammatory status in patients with GDM.

Real-time reverse-transcriptase Polymerase Chain Reaction (RT) PCR gene expression method was chosen as it quantitates the initial amount of the template most specifically, sensitively and reproducibly, and is a preferable alternative to other forms of quantitative RT-PCR that detect the amount of final amplified product at the end-point⁴⁵⁻⁴⁷.

In the present study it was observed that the gestational diabetes mellitus GDM group had a higher mean TNF- α level when compared to the control group at baseline before initial periodontal therapy and 2 months after the completion of Phase I therapy which supports the hypothesis of an association between periodontal disease and gestational diabetes mellitus GDM⁴⁸⁻⁵⁰. Hence, we can conclude that the local inflammatory changes that have resulted in elevated levels of TNF- α in GCF might have contributed to the increased levels of TNF- α in serum i.e., systemic "Spill" of cytokine via the circulation as reported by *Offenbacher*⁵¹.

*Goktas et al.*⁵² concluded that obesity is a chronic low-grade inflammatory disease characterized by overproduction of inflammatory adipokines by adipose tissue and this may be the link between obesity, Cardiovascular diseases (CVD) and diabetes. One of these adipokines is Tumor Necrosis Factor Alpha (TNF- α)⁵³⁻⁵⁶ contributing to the pathogenesis of metabolic syndrome, insulin resistance, type 2 diabetes, and cardiovascular disease.

Gestational diabetes mellitus GDM has been considered a great risk for developing more severe periodontal disease. It is important to clarify that gestational diabetes mellitus GDM does not exclude the possibility that unrecognized glucose intolerance may be present prior to pregnancy⁵⁷⁻⁶¹. Therefore, a probable undiagnosed case of hyperglycemia could be responsible for the increased level of periodontal disease in our GDM group (group 1).

The observed association between periodontal disease and gestational diabetes mellitus GDM might be explained by gestational diabetes mellitus

GDM causing periodontitis, similar to type 1 or 2 diabetes where long duration of elevated blood glucose levels (hyperglycemia), impaired insulin resistance, vascular changes, altered oral microflora, abnormal collagen metabolism, and the consequent hyperglycemia and hyperlipidemia of diabetes result in metabolic alterations which then exacerbate the bacteria-induced inflammatory periodontitis⁶².

However, compared to type 1 or 2 diabetes, gestational diabetes mellitus GDM only represents an early stage of glucose dysregulation and a temporary impaired glucose tolerance that occur in later pregnancy. The elevated glucose levels in the majority of women diagnosed with gestational diabetes mellitus GDM will usually return to normal after birth⁶³. Therefore, the hyperglycemia of gestational diabetes mellitus GDM may be too mild and of too short duration to have a significant effect on gingival tissues and to cause a destruction of the supporting structures of the teeth manifested as periodontitis.

An alternative explanation is that periodontal disease may be a cause, instead of the result of gestational diabetes mellitus GDM. Periodontal infection, a local and chronic sub-clinical inflammation, triggers a maternal systemic inflammatory response. Since pregnancy itself is a stressful state with increased inflammatory activity and marked insulin resistance, such an infection-induced insulin resistance in response to maternal periodontal infection may thus worsen the preexisting pregnancy-induced insulin resistance that may cause impaired glucose tolerance and the manifestation of gestational diabetes mellitus GDM⁶⁴.

There may be a common genetic cause for both periodontal disease and gestational diabetes mellitus GDM that results in the observed association between the two disorders. Although there is lack of clear correlation between the gene polymorphisms and GDM, a few studies suggested that cytokines such as TNF- α , IL-6, and IL-1 polymorphisms may

be associated with the risk of insulin resistance or type 2 diabetes as well as periodontal disease⁶⁵⁻⁶⁷. Therefore, there is a possibility that pre-existing genetic polymorphisms result in imbalances between the pro vs. anti-inflammatory cytokine systems predisposing to both periodontal disease and gestational diabetes mellitus GDM simultaneously. However, the results of the study conducted by *Mishra et al.*⁶⁸ showed that periodontal disease is not significantly associated with gestational diabetes mellitus GDM.

In this study, the systemic reduction of TNF- α level was positively correlated with reduction of Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), and Clinical Attachment Level (CAL) that are important inflammatory indices of periodontitis, which might be part of the proof of the mechanism that links the focal infection with systemic cytokine levels.

*ZHOU et al.*⁶⁹ reported that the systemic reduction of TNF- α level was positively correlated with reduction of bleeding index (BI) and plaque index (PI), indicating that local periodontal treatment could reduce the systemic cytokines level in chronic periodontitis subjects with chronic heart disease.

The possible mechanisms that connect focal infection in periodontitis with systemic cytokine levels might be that the TNF- α stimulated the expression of interleukin 6 (IL-6), which consequently augmented the C-reactive protein (CRP) gene expression in the liver^{70,71}.

Finally, we can conclude that the Improvement of periodontal condition is found to be associated with reduction in TNF- α levels and that phase 1 periodontal therapy for gestational diabetic females is a safe & effective line of treatment. More research on the biological effects of TNF- α should be considered and further studies are needed to understand the role of TNF- α in periodontal health and disease.

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