

Salivary Adiponectin and Leptin Levels are Increased in Women with Gestational Diabetes Mellitus and Gingival Inflammation

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Purpose: Gestational diabetes mellitus (GDM) is defined as glucose intolerance with first onset or diagnosis in pregnancy. This study evaluated clinical and biochemical parameters in a possible association between GDM and gingival inflammation.

Materials and Methods: A total of 87 pregnant women – 44 with GDM and 43 without (NGDM) – were included. Subgroups were created according to gingival inflammation. Plaque index (PI), bleeding on probing (BOP), and probing depth (PD) were recorded.

Results: Age, anthropometric variables and baby weight (g) were all statistically significantly higher in the GDM group ($p < 0.0001$). Systolic and diastolic blood pressure (mmHg), saliva, serum leptin and adiponectin levels were similar in the GDM and NGDM groups ($p = 0.605$, $p = 0.662$, $p = 0.737$, and $p = 0.596$, respectively). Salivary adiponectin levels were statistically significantly higher in the two subgroups with gingivitis compared to those with clinically healthy periodontium ($p < 0.01$). Serum adiponectin levels were statistically significantly higher in the NGDM subgroup with gingivitis than the NGDM group with clinically healthy periodontium ($p < 0.05$). Statistically significant positive correlations were found between PD, PI, BOP and saliva adiponectin levels in the GDM group ($p < 0.05$). Positive correlations were also found between clinical periodontal parameters and saliva, serum levels of adiponectin in the control group without GDM ($p < 0.05$).

Conclusion: The higher salivary adiponectin levels in the gingivitis groups suggest that gingival inflammation is more likely to influence local inflammatory parameters both in the presence and absence of GDM. Further larger-scale studies are required to better clarify the possible interactions between gingival inflammation and GDM.

Key words: adiponectin, gestational diabetes, gingivitis, MeSH key words, leptin, saliva

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Gingivitis is characterised by reddened gingiva, an increased tendency for bleeding and increased probing depth due to the presence of oedema. Chronic infectious diseases, e.g. periodontal diseases including gingivitis, can cause a persistent inflammatory state. A close relationship has been reported between pregnancy and periodontal dis-

eases, and pregnancy may increase preexisting periodontal inflammatory response.²²

Gestational diabetes mellitus (GDM) is defined as varying glucose intolerance with first onset or diagnosis in pregnancy.⁹ GDM represents a failure of the pancreas to appropriately increase β -cell mass³² and insulin secretion⁹ as a

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response to the progressive insulin resistance of the latter stages of gestation. Approximately 4%-10% of all pregnant women have GDM, and it is associated with statistically significantly increased risks of maternal and infant morbidity.¹ Pancreatic β -cell destruction can result from the pro-inflammatory imbalance created by a sustained elevation of cytokines.²⁶ Current evidence shows that diabetes mellitus (DM) causes more rapid and severe periodontal tissue destruction; further, a bidirectional relationship between periodontal disease and type-2 DM has been suggested.¹⁰ To date, few studies have examined a possible relationship between periodontal disease and GDM.^{37,38,40} Viable bacteria, bacterial products from the microbial dental plaque and pro-inflammatory cytokines secreted by the inflamed periodontal tissues can enter the circulation and trigger a maternal systemic inflammatory response.^{2,21}

Leptin and adiponectin are adipokines that are secreted primarily by adipose tissues and are not only involved in energy storage but also have an endocrine function.^{13,30} Leptin plays a key role in the regulation of energy intake and energy expenditure. It also regulates T lymphocyte proliferation, activation and cytokine production; elevated levels of leptin are present during infection and inflammation.^{11,24,43} Adiponectin is associated with modulation of inflammatory responses, via attenuation of tumor necrosis factor- α (TNF- α) effects^{41,44} and inhibition of nuclear factor kappa B and interleukin (IL)-6 production, as well as induction of anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist.³⁶

Adiposity is an important modifiable risk factor for the development of GDM.⁶ Increased leptin levels may contribute to insulin resistance in GDM.¹² Adiponectin is thought to have beneficial effects on insulin sensitivity,⁴² and administration of adiponectin to diabetic mice has been shown to enhance insulin activity.⁷ Several studies have reported an association between severity of periodontitis and leptin levels in serum or gingival crevicular fluid (GCF).^{8,19,20} Increased circulating levels of leptin, TNF- α C-reactive protein (CRP) and decreased levels of adiponectin have been reported in women with GDM.²⁵ However, the possible relationship between GDM, gingival inflammation and local, systemic levels of adiponectin or leptin has not yet been investigated.

It is hypothesised that chronic gingival inflammation can induce a sustained systemic inflammatory response, which can have a confounding effect in pregnancy-induced insulin resistance. Additionally, GDM may exacerbate clinical and/or biochemical findings of gingival inflammation. Therefore, the aim of the present study was to evaluate a possible interaction between GDM and gingival inflammation via local and systemic levels of leptin and adiponectin.

MATERIALS AND METHODS

Study Population

Forty-four pregnant women with GDM (aged 26.0 to 38 years) and 43 counterparts without GDM (aged 27 to 36 years) were

recruited from September 2012 to March 2013 at the Endocrinology and Metabolism outpatient clinic, Aydın State Hospital, Aydın, Turkey. The study was conducted in full accordance with ethical principles, including the World Medical Association's Declaration of Helsinki, as revised in 2008. The study protocol was approved by the Ethics Committee of the Medical Faculty of Ege University (protocol number 13-2/9). The study protocol was explained, and written informed consent was received from each individual before enrolment in the study. The study conforms to STROBE guidelines for observational studies.³⁵ Medical and dental histories were obtained from each individual, and saliva and serum samples were obtained before clinical periodontal examination. The inclusion criteria were being in the third trimester, not having any other diagnosed systemic diseases requiring medications, such as antibiotics, nonsteroidal anti-inflammatory drugs, corticosteroids or drugs that cause gingival overgrowth. Smoking status was determined according to the self-reports of the pregnant women. The exclusion criteria were having a body mass index (BMI) value ≥ 30 (kg/m²), being a current smoker or having been a smoker before pregnancy.

American Diabetes Association criteria were used for clinical diagnosis. Women at 24-28 weeks of gestation not previously diagnosed with overt diabetes and participants with a 75-g oral glucose tolerance test (OGTT) with plasma glucose measurement fasting and at 1 h and 2 h were diagnosed as GDM.³ GDM was based on only one blood glucose value above the specified cut-off points (fasting: ≥ 92 mg/dl [5.1 mmol/l], 1 h: ≥ 180 mg/dl [10.0 mmol/l], 2 h: ≥ 153 mg/dl [8.5 mmol/l]). All pregnant women known not to have prior diabetes and not meeting the diagnostic criteria for GDM were assigned to the non-GDM (without GDM) (NGDM) control group.

Diagnosis of gingivitis was assigned when bleeding on probing (BOP) was present at $> 50\%$ of all sites² and probing depth (PD) was < 3 mm at 90% of the measured sites, but no more than one site had a PD > 4 mm and clinical attachment level (CAL) of 1 mm, and no clinical sign of periodontitis was evident.⁴ All participants had at least 20 teeth present. The periodontally healthy females had BOP at $< 30\%$ of the measured sites and no clinical sign of alveolar bone loss.^{2,15}

Saliva Sampling

Whole saliva samples were obtained simply by having the participants expectorate into polypropylene tubes prior to the clinical periodontal measurements or any periodontal intervention, and in the morning following an overnight fast during which participants were requested not to drink (except water) or chew gum.²⁸ The samples were then immediately frozen and stored at -40°C until the sample collection period was completed. Samples were thawed immediately before assays.

Serum Sampling

Five millilitres of venous blood were taken from the antecubital vein by a standard venipuncture method, and serum was separated from blood by centrifugation at $1500 \times g$ for 10 min. The serum samples were then stored at -40°C until

Table 1 Clinical periodontal measurements and anthropometric parameters of the GDM and NGDM groups

	GDM N=44	NGDM N=43	
Clinical variable	Mean ± SD	Mean ± SD	p
Age (years)	32.91 ± 4.5	27.92 ± 4.7	< 0.0001
PD (mm)	2.1 ± 1.1	1.8 ± 0.9	= 0.137
PI (%)	53.07 ± 29.1	45.07 ± 27.1	= 0.169
BOP (%)	53.01 ± 29.07	43.02 ± 26.03	= 0.106
Gingivitis/healthy	24/20	22/21	
Anthropometric parameters	Mean ± SD	Mean ± SD	p
Pregestation weight (kg)	71 ± 15	57 ± 7	< 0.001
BMI (kg/m ²)	29.4 ± 4.4	21.5 ± 2.1	< 0.001
Fasting glucose (mmol/l)	91.3 ± 14	82.3 ± 8	< 0.001
1. hour (mmol/l)	202 ± 21	161 ± 14	< 0.001
2. hour (mmol/l)	176 ± 21	130 ± 16	< 0.001
Fasting insulin (μUL/m)	14.5 ± 3.51	11.6 ± 1.98	< 0.001
HOMA-IR	3.27 ± 0.99	2.33 ± 0.44	< 0.001
Baby weight (g)	3373.69 ± 425	3193 ± 288	0.008
Systolic blood pressure (mmHg)	116.1 ± 11.18	117.1 ± 9.3	0.792
Diastolic blood pressure (mmHg)	71 ± 9	70 ± 9	0.691
GDM: gestational diabetes mellitus; NGDM: without GDM; PD: pocket depth; PI: plaque index; BOP: bleeding on probing; BMI: body mass index. Age was statistically significantly higher in the GDM group. All parameters apart from the systolic and diastolic blood pressure values showed statistically significant differences between the study groups in anthropometric parameters.			

subsequent biochemical analyses and thawed immediately before assaying.

Clinical Periodontal Measurements

Before GCF sampling, clinical periodontal recordings were performed using a Williams periodontal probe (Hu-Friedy; Chicago, IL, USA) at four sites (mesio-buccal, mid-buccal, disto-buccal, mid-lingual) of each tooth except the third molars, and included the following: 1) PD; 2) PI as present or absent; and 3) BOP as present or absent. BOP was deemed positive if it occurred within 15 s after periodontal probing. Visible plaque accumulation was recorded dichotomously by visual examination.²⁹ The measurements were conducted by periodontists who were trained and calibrated initially and every 6 months during the study period (ÖÖ, AA, PG). The endocrinologist (BC-O) knew whether the pregnant woman had gestational diabetes or not, and the three periodontists were blinded to the study group during the clinical periodontal evaluation.

Measurement of Leptin and Adiponectin in Saliva and Serum Samples

Commercial enzyme-linked immunosorbent assay (ELISA) kits were purchased for the measurement of leptin and adi-

ponectin (R&D Systems; Minneapolis, MN, USA), and the analyses were performed according to the manufacturer's instructions.

Statistical Analysis

Sample size analysis of the present study was based on a pilot experiment in which adiponectin concentrations were measured and a 15% difference between two groups in adiponectin concentrations was obtained. These results were used for statistical power calculations. An adjustment to type-1 error ($\alpha = 0.05$) was made to allow for comparisons among groups. Statistical power exceeded 80% when the average number in each group was 20. The distribution of the data was validated using the D'Agostino-Pearson omnibus normality test, and statistical analysis was performed using non-parametric methods. Comparisons between the study groups and subgroups were made using the Kruskal-Wallis test. Statistical analyses were conducted using statistical software (GraphPad Prism version 6.00c for Mac OS X, GraphPad Software; La Jolla, CA, USA), and statistical significance was set at $p < 0.05$. Correlations between parameters were analyzed by Spearman's correlation test.

RESULTS

Clinical periodontal measurements revealed similar findings in the study groups ($p > 0.05$) (Table 1). The mean maternal age of the women in the GDM group was statistically significantly higher than that of the NGDM group (32.91 and 27.92 years, respectively, $p < 0.0001$), with median values of 33 years in the GDM group and 26.82 years in the NGDM group. The number of patients with gingivitis was similar in the groups.

Anthropometric parameters are also shown in Table 1. Pre-gestation weight (kg), BMI (kg/m^2), fasting glucose (mmol/l), 1-h and 2-h blood glucose levels (mmol/l), fasting insulin ($\mu\text{UL}/\text{ml}$), HOMA-IR (Homeostatic Model of Assessment-Insulin Resistance) and birth weight (g) were all statistically significantly higher in the GDM group than in the controls ($p < 0.0001$). Systolic and diastolic blood pressure (mmHg) levels were similar in the study groups.

Saliva and serum leptin as well as adiponectin levels were similar in the GDM and NGDM groups ($p = 0.605$, $p = 0.662$, $p = 0.737$, and $p = 0.596$, respectively) (Table 2). When the study groups were divided into subgroups regarding with the presence or absence of gingivitis, serum and saliva leptin levels revealed similar values ($p > 0.05$) (Table 3). Salivary adiponectin levels were statistically significantly higher in the two subgroups with gingivitis (GDM with gingivitis and NGDM with gingivitis) compared to those with clinically healthy periodontium ($p < 0.01$). Serum adiponectin level was statistically significantly higher in the NGDM subgroup with gingivitis than the NGDM group with clinically healthy periodontium ($p < 0.05$).

Correlations between the clinical and biochemical data are presented in Tables 4 and 5. Statistically significant positive correlations were found between PD, PI, BOP and saliva adiponectin levels in the GDM group ($p < 0.05$). Positive correlations were also found between the clinical periodontal parameters and saliva, serum levels of adiponectin in the NGDM group ($p < 0.05$). There were no correlations between HOMA-IR and saliva, serum levels of leptin and adiponectin in the GDM or NGDM groups ($p > 0.05$).

DISCUSSION

The aim of the present study was to compare the clinical periodontal findings as well as the serum and saliva levels of leptin and adiponectin between women with or without GDM. Smoking, obesity and DM are among the major risk factors for periodontal diseases. Therefore, non-obese and non-smoking women were included in the present study. To the best of our knowledge, this is the first study comparing salivary and serum levels of leptin and adiponectin in pregnant women with or without GDM. According to the present findings, saliva and serum leptin and adiponectin levels were similar in women with GDM and their non-GDM counterparts.

Pregnancy is not a primary cause of periodontal disease but rather an exacerbating factor for pre-existing inflammatory periodontal conditions.²² The newly developed peri-

odontal pockets resolve after delivery, and maintenance of good periodontal health during pregnancy has been suggested as a therapeutic goal.²⁷ In a case control study, Xiong et al³⁹ reported that the presence of at least one site with BOP was more frequent and the mean PD was higher in women with GDM compared to non-GDM controls. However, the authors did not provide any information on plaque scores.³⁹ In the present study, PD, BOP and plaque scores were similar in the study groups.

Advanced maternal age is associated with adverse pregnancy outcomes, including GDM. Lui et al²³ stated that maternal risks are higher during the adolescent period but particularly at advanced maternal ages above 40 years. The mean maternal age in the present GDM group was higher than that in the NGDM group, but none of the participants were older than 35 years. Therefore, this age difference between the study groups is not likely to have played a role in the presence or absence of GDM.

In a previous study, serum samples were taken from 33 patients with chronic periodontitis and 18 healthy individuals.³³ The serum leptin and adiponectin levels were measured before and after nonsurgical periodontal treatment, and statistically significant differences were found between the healthy control and periodontitis groups. The serum leptin level was associated with mean probing depth, mean clinical attachment level, mean alveolar bone loss, and BMI. Nonsurgical periodontal treatment resulted in statistically significant decreases in serum leptin levels.³³ In a study with four groups of individuals with a. normal weight and no periodontitis ($n = 20$), b. normal weight and chronic periodontitis ($n = 20$), c. obesity and no periodontitis ($n = 18$) and d. obesity and chronic periodontitis ($n = 20$), serum and gingival crevicular fluid levels of adipocytokines were evaluated both in shallow and deep sites.⁴⁵ Adiponectin levels were lower in the periodontitis than in non-periodontitis groups, whereas the normal weight non-periodontitis group presented the lowest serum leptin levels. Those authors concluded that periodontitis mainly influenced the circulating levels of adiponectin, and both obesity and periodontitis affected the circulating levels of leptin in favour of pro-inflammation.⁴⁵ In the present study, saliva and serum leptin as well as adiponectin levels in the GDM and NGDM groups were not statistically significantly different. This discrepancy between the studies can be explained by the different clinical periodontal diagnoses of the study groups. But when our study groups were further divided into subgroups according to the presence or absence of gingivitis, the two subgroups with gingivitis exhibited statistically significantly higher salivary adiponectin levels than those with clinically healthy periodontium. Serum adiponectin levels were also higher in the two subgroups with gingivitis, but the difference reached the level of statistical significance only in the NGDM group. Adiponectin has anti-inflammatory functions, via inhibition of tumor necrosis factor- α (TNF- α),^{41,44} nuclear factor kappa B and interleukin (IL)-6 production.³⁶ Chronic infectious diseases such as gingivitis increase the inflammatory cytokine levels. The increased level of adiponectin in the present gingivitis groups may be

Table 2 Salivary and serum levels of leptin and adiponectin in the GDM and NGDM groups

Saliva data	GDM N=44	NGDM N=43	p
	Mean ± SD	Mean ± SD	
Leptin (ng/ml)	0.423 ± 0.140	0.463 ± 0.203	0.605
Adiponectin (ng/ml)	12.94 ± 12.4	10.75 ± 9.3	0.662
Serum data			
Leptin (ng/ml)	14.91 ± 11.55	15.61 ± 11.68	0.737
Adiponectin (ng/ml)	11.46 ± 5.07	12.37 ± 9.9	0.596
GDM: gestational diabetes mellitus; NGDM: without GDM. There were no statistically significant differences between the study groups.			

Table 3 Biochemical data in the subgroups with or without gingival inflammation

	GDM N=44			NGDM N=43		
	With gingivitis N=24	Periodontally healthy N=20	p	With gingivitis N=22	Periodontally healthy N=21	p
Saliva						
Leptin (ng/ml)	0.39 ± 0.06	0.46 ± 0.20	0.803	0.40 ± 0.07	0.51 ± 0.25	0.101
Adiponectin (ng/ml)	17.11 ± 13.93†	6.924 ± 6.19	0.001	13.57 ± 10.14*	7.98 ± 7.18	0.008
Serum						
Leptin (ng/ml)	13.98 ± 11.96	16.25 ± 11.12	0.340	15.46 ± 10.74	15.75 ± 12.60	0.801
Adiponectin (ng/ml)	12.07 ± 5.83	10.58 ± 3.69	0.604	14.70 ± 13.34*	9.78 ± 2.57	0.034
GDM: gestational diabetes mellitus; NGDM: without GDM. *Statistically significantly higher in the NGDM gingivitis than NGDM periodontally healthy. † Statistically significantly higher in the GDM gingivitis than GDM periodontally healthy.						

Table 4 Correlation between the clinical periodontal parameters and the biochemical data in the GDM group

Biochemical data		Clinical periodontal parameters		
		PD (mm)	PI (%)	BOP (%)
Salivary leptin	r	0.067	0.022	0.029
	p	0.664	0.886	0.851
Salivary adiponectin*	r	0.464	0.483	0.492
	p	0.001	0.001	0.001
Serum leptin	r	-0.056	-0.020	-0.030
	p	0.713	0.892	0.844
Serum adiponectin	r	0.082	0.099	0.082
	p	0.593	0.521	0.595
PD: pocket depth; PI: plaque index; BOP: bleeding on probing. *Saliva adiponectin levels were positively correlated with the clinical periodontal parameters.				

Table 5 Correlation between the clinical periodontal parameters and the biochemical data in the NGDM group

Biochemical data		Clinical periodontal parameters		
		PD (mm)	PI (%)	BOP (%)
Salivary leptin	r	-0.237	-0.257	-0.215
	p	0.124	0.096	0.164
Salivary adiponectin*	r	0.547	0.487	0.508
	p	0.001	0.001	0.001
Serum leptin	r	-0.082	-0.048	-0.056
	p	0.598	0.759	0.720
Serum adiponectin*	r	0.339	0.360	0.363
	p	0.026	0.017	0.016
PD: pocket depth; PI: plaque index; BOP: bleeding on probing. *Salivary and serum levels were positively correlated with the clinical periodontal parameters.				

explained by a possible attempt of the host response to compensate for the already increased inflammatory cytokine production.

These statistically significant differences together with the positive correlations between PD, PI, BOP and saliva adiponectin levels in the GDM group and the positive correlations between the clinical periodontal parameters and both saliva and serum levels of adiponectin in the NGDM group provide further support for the association of adiponectin and gingival inflammation.

In another study, 24 obese and 24 normal-weight patients all with chronic periodontitis received nonsurgical periodontal therapy.¹⁷ Serum levels of leptin and adiponectin were evaluated at baseline and after completion of nonsurgical periodontal treatment. Serum leptin levels were higher in the obese patients than in the normal-weight counterparts. Serum levels of leptin and adiponectin were similar in the obese and normal-weight groups after nonsurgical periodontal therapy.¹⁷ Kardesler et al¹⁸ investigated the serum leptin and adiponectin levels in 13 well-controlled (glycated hemoglobin [HbA1c] <7%) and 12 poorly controlled (HbA1c ≥7%) patients with type 2 diabetes and 15 systemically healthy patients all with chronic periodontitis. They found that adiponectin levels decreased significantly in the well-controlled diabetic group 3 months after the nonsurgical periodontal treatment but increased in the systemically healthy group. Leptin levels were similar at all times in the study groups. After completion of the nonsurgical periodontal treatment, leptin levels significantly increased at the 1-month control in the well-controlled diabetic group, and decreased at the 3-month control in the systemically healthy group.¹⁸

A meta-analysis of 9 prospective studies on adiponectin and 8 prospective studies on leptin indicated that adiponectin levels during the first or second trimester of pregnancy were lower in pregnant women who later developed GDM compared to the women without GDM, whereas leptin levels were higher.⁵ In our study, serum leptin and adiponectin levels were similar in the study groups. Larger-scale and prospective studies with assessment of adipokines during pregnancy are needed to understand the trajectories and dynamic associations of adipokines with GDM risk.⁵

The results of a study investigating insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR) indicated a statistically significant correlation between the HOMA-IR and leptin concentration, but not between the HOMA-IR and adiponectin concentration.³⁴ There was a significant inverse correlation between the HOMA-IR and adiponectin:leptin ratio.³⁴ In the present study, we could not find any correlation between HOMA-IR and serum or saliva leptin and adiponectin levels in the GDM and NGDM groups.

GDM is an inflammatory condition and various cytokines have been implicated in its physiopathology, but their role has not yet been clarified.^{16,31} Hyperglycemia in GDM is transient and of short duration, and this may not persist long enough to initiate or exacerbate periodontal tissue destruction. Therefore, periodontal diseases have been specu-

lated to be a cause rather than a result of GDM.^{39,40} Locally produced inflammatory cytokines and bacterial products trigger systemic inflammation, which may eventually destroy pancreatic β -cells.^{39,40} Periodontal diseases existing before the onset of pregnancy can increase the prevalence of insulin resistance and lead to GDM during pregnancy. Accordingly, the clinical severity of gingival inflammation has been correlated with sex steroid hormone levels during pregnancy.^{14,39}

CONCLUSION

The present finding of similar clinical periodontal data in women with or without GDM is likely to explain the similar serum and salivary levels of adiponectin and leptin in these groups. On the other hand, the higher salivary adiponectin levels in the two subgroups with gingivitis suggest that adiponectin acts as an anti-inflammatory mediator in chronic gingival inflammation, and it is more likely to influence local inflammatory parameters. Future studies on a larger scale as well as periodontal intervention may help to better understand the possible relationship between adipokines and inflammatory periodontal diseases and GDM.

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REFERENCES

1. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists. *Obstet Gynecol* 2001;98:525–538.
2. Amar S, Han X. The impact of periodontal infection on systemic diseases. *Med Sci Monitor* 2003;9:RA291–RA299.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013;36 (Suppl. 1):S67–S74.
4. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* 1999;4:1–6.
5. Bao W, Baecker A, Song Y, Kiely M, Liu S, Zhang C. Adipokine levels during the first or early second trimester of pregnancy and subsequent risk of gestational diabetes mellitus: A systematic review. *Metabolism* 2015; 64:756–764.
6. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with type 2 diabetes. *Diabet Med* 2004;21: 103–113.
7. Bergand AH, Scherer PE. Adipose tissue, inflammation and cardiovascular disease. *Circulation Research* 2005;96:939–949.
8. Bozkurt FY, Ay ZY, Sutcu R, Delibas N, Demirel R. Gingival crevicular fluid leptin levels in periodontitis patients with long-term and heavy smoking. *J Periodontol* 2006;77:634–640.
9. Buchanan TA, Xiang A, Kjos SL, Watanabe R. What is gestational diabetes? *Diabetes Care* 2007;Suppl 30:S105–111.
10. Chapple IL, Genco R. Working group 2 of joint EFP/AAP workshop. Diabetes and periodontal diseases: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. *J Clin Periodontol* 2013;Suppl 14:106–112.

11. Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS. Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. *Diabetes* 1998;47(2):239–243.
12. Cseh K, Baranyi E, Melczer Z, Speer G, Kovács M, Gerő G, et al. The pathophysiological influence of leptin and the tumor necrosis factor system on maternal insulin resistance: negative correlation with anthropometric parameters of neonates in gestational diabetes. *Gynecol Endocrinol* 2002;16:453–460.
13. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115:911–919.
14. Figuero E, Carrillo-de-Albornoz A, Martin C, Tobias A, Herrera D. Effect of pregnancy on gingival inflammation in systemically healthy women: a systematic review. *J Clin Periodontol* 2013;40:457–473.
15. Garcia RI, Henshaw MM, Krall EA. Relationship between periodontal disease and systemic health. *Periodontol* 2000 2001;25:21–36.
16. Gomes CP, Torloni MR, Gueuvoghlian-Silva BY, et al. Cytokine levels in gestational diabetes mellitus: a systematic review of the literature. *Am J Reprod Immunol* 2013;69:545–557.
17. Gonçalves TE, Feres M, Zimmermann GS, Faveri M, Figueired LC, Braga PG, Duarte PM. Effects of scaling and root planing on clinical response and serum levels of adipocytokines in patients with obesity and chronic periodontitis. *J Periodontol* 2015;86:53–61.
18. Kardeşler L, Buduneli N, Cetinkalp S, Kinane DF. Adipokines and inflammatory mediators after initial periodontal treatment in patients with type 2 diabetes and chronic periodontitis. *J Periodontol* 2010;81:24–33.
19. Karthikeyan BV, Pradeep AR. Gingival crevicular fluid and serum leptin: Their relationship to periodontal health and disease. *J Clin Periodontol* 2007;34:467–472.
20. Karthikeyan BV, Pradeep AR. Leptin levels in gingival crevicular fluid in periodontal health and disease. *J Periodontol Res* 2007;42:300–304.
21. Kinane DF. Causation and pathogenesis of periodontal disease. *Periodontol* 2000 2001;25:8–20.
22. Laine MA. Effect of pregnancy on periodontal and dental health. *Acta Odontol Scand* 2002;60: 257–264.
23. Liu X, Zhang W. Effect of maternal age on pregnancy: a retrospective cohort study. *Chin Med J* 2014;127:2241–2246.
24. Maruna P, Gurlich R, Frasko R, Haluzik M. Serum leptin levels in septic men correlate well with C-reactive protein (CRP) and TNF-alpha but not with BMI. *Physiol Res* 2001;50:589–594.
25. Meller M, Qiu C, Vadachkoria S, Abetew D, Luthy D, Williams M. Changes in placental adipocytokine gene expression associated with gestational diabetes mellitus. *Physiol Res* 2006;55:501–512.
26. Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. *Trends Endocrinol Metab* 2000;1:212–217.
27. Moss KL, Beck JD, Offenbacher S. Clinical risk factors associated with incidence and progression of periodontal conditions in pregnant women. *J Clin Periodontol* 2005;32:492–498.
28. Navazesh M. Methods for collecting saliva. *Ann N Y Acad Sci* 1993;694: 72–77.
29. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol* 1972;43:38.
30. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11(2):85–97.
31. Richardson AC, Carpenter MW. Inflammatory mediators in gestational diabetes mellitus. *Obstet Gynecol Clin North Am* 2007;34:213–224.
32. Rieck S, Kaestner KH. Expansion of beta-cell mass in response to pregnancy. *Trends Endocrinol Metab* 2010;21:151–158.
33. Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Su-gita N, Yoshie H. The effect of periodontal treatment on serum leptin, interleukin-6, and C-reactive protein. *J Periodontol* 2010;81:1118–1123.
34. Skvarca A, Tomazic M, Blagus R, Krhin B. and Janez, A. Adiponectin/leptin ratio and insulin resistance in pregnancy. *J Int Med Res* 2013;41: 123–128.
35. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *PLoS Med* 2007;4:e296.
36. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* 2004;323:630–635.
37. Xiong X, Buekens P, Fraser W, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: A systematic review. *BJOG* 2006;113:135–143.
38. Xiong X, Buekens P, Vastardis S, Yu SM. Periodontal disease and pregnancy outcomes: State-of-the-science. *Obstet Gynecol Surv* 2007;62: 605–615.
39. Xiong X, Elkind-Hirsch KE, Vastardis S, Delarosa RL, Pridjian G, Buekens P. Periodontal disease is associated with gestational diabetes mellitus: A case-control study. *J Periodontol* 2009;80:1742–1749.
40. Xiong X, Elkind-Hirsch KE, Xie Y, Delarosa R, Maney P, Pridjian G, et al. Periodontal disease as a potential risk factor for the development of diabetes in women with a prior history of gestational diabetes mellitus. *J Public Health Dent* 2013;73:41–49.
41. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and non-alcoholic fatty liver diseases in mice. *J Clin Invest* 2003;112(1):91–100.
42. Yamauchi T, Kamon J, Minokoshi Y et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine* 2002;8(11):1288–1295.
43. Yılmaz Z, Ilcol YO, Ulus IH. Endotoxin increases plasma leptin and ghrelin levels in dogs. *Crit Care Med* 2008;36:828–833.
44. Yokota T, Oritani K, Takahashi I, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000;96:1723–1732.
45. Zimmermann GS, Bastos MF, Dias Gonçalves TE, Chambrone L, Duarte PM. Local and circulating levels of adipocytokines in obese and normal weight individuals with chronic periodontitis. *J Periodontol* 2013;84:624–633.