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ORIGINAL ARTICLE

Early pregnancy levels of gingival crevicular fluid matrix metalloproteinases-8 and −9 are associated with the severity of periodontitis and the development of gestational diabetes mellitus

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Abstract

Background: Gestational diabetes mellitus (GDM) is increasing worldwide and women with a history of GDM are at risk of developing type 2 diabetes which is a risk factor for periodontitis.

Aim: To explore the association between the concentrations of matrix metalloproteinase (MMP)-8 and −9 in gingival crevicular fluid (GCF) during early pregnancy with the periodontal diagnosis and the risk of GDM development.

Materials and methods: A prospective cohort study, including 314 women, enrolled at 11 to 14 weeks of pregnancy was conducted. A complete maternal/obstetric and periodontal exam was performed, and GCF samples were obtained for the MMP-8 and −9 determination by Multiplex Elisa Assays. Mann-Whitney test; Spearman's correlation and log-binomial regression model estimated the association between MMPs concentration in GCF and GDM.

Results: Fourteen percent of the pregnancies were diagnosed with GDM. An increase in the concentration of MMP-8 and −9 in women with periodontitis stage III and IV compared to periodontitis stage I was observed (99.31 ng/mL [IQR: 85.32] versus 71.95 ng/mL [IQR: 54.04], and 262.4 ng/mL [IQR: 312.55] versus 114.1 ng/mL [IQR: 184.94], respectively). Women who developed GDM showed increased concentrations of MMP-8 and −9 in GCF since the beginning of pregnancy ($P = 0.0381$; $P = 0.0302$, respectively). MMP-8 concentration in GCF was associated with GDM (RR: 1.19; $P = 0.045$; CI 95% 1.00 to 1.40; and RR: 1.20; $P = 0.063$; CI 95% 0.99 to 1.45 in the adjusted model).

Conclusion(s): GCF concentrations of MMP-8 and −9 at early of pregnancy are increased in women with severe periodontitis and associated with the GDM development.

KEYWORDS
biomarkers, diabetes, gestational, gingival crevicular fluid, metalloproteinases, periodontitis

1 | INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as glucose intolerance identified for the first time during pregnancy.[1-4] Over the past 20 years, the prevalence of GDM has increased (WHO, 2016),[5] and some of the contributing factors to this emerging public health problem are population aging, urbanization, obesity, sedentary lifestyle and stressful modern life.[6,7] Women with GDM experience an increased risk of developing other pregnancy complications, such as preeclampsia (PE), and their offspring are at higher risk of developing short-term adverse outcomes including macrosomia, neonatal hypoglycemia, and neonatal cardiac dysfunction, as well as long term complications, such as obesity, impaired glucose tolerance, and diabetes in adolescence or early adulthood.[8-10] Finally, different studies have demonstrated that as high as 50% of women diagnosed with this metabolic disorder are expected to develop Type 2 Diabetes Mellitus (T2DM) within 10 to 30 years.[9-11]

In the past few years, periodontitis has emerged as a potential risk factor for GDM.[12-14] Conversely, studies are supporting that women with GDM are at greater risk for developing more severe periodontal disease than pregnant women without GDM.[12,13] Although the exact mechanism involved in this association remains unclear,[14,15] the fact that periodontitis can contribute to the systemic spread of bacteria and bacterial products, and that may subsequently induce a systemic inflammatory process that is related to the physiopathology of GDM, makes this association biologically plausible.[16] On the other hand, it is well known that hormonal changes that occur during pregnancy increase the susceptibility of periodontal tissues to develop inflammation.[17-19] Moreover, a solid body of evidence has demonstrated that the extent of gingival inflammation increases during pregnancy and cross-sectional studies indicate that almost all pregnant women develop gingivitis between their 2nd to 3rd trimester of gestation, with a gradual decrease during the postpartum period.[17-20]

During pregnancy, many environmental changes occur that may promote periodontal tissue breakdown which has been endorsed to changes in estrogen and progesterone concentrations.[17,20] Periodontal destruction and gingival inflammation during pregnancy may also be exacerbated by the overexpression of matrix metalloproteinases (MMPs), which can degrade most extracellular matrices.[21,22] Previous studies have established that concentrations of serum and oral MMP-8 and MMP-9 are correlated with clinical periodontal parameters in a dose-dependent manner in patients with periodontitis, even after adjusting for potential confounders.[23-25] Nevertheless, the expression of MMP-8 and −9 in periodontal tissues and their role in pregnancy have been poorly explored and the results are not conclusive.[21,22] On the other hand, different studies have shown that in gingiva and oral fluids of non-pregnant diabetic patients, the MMP-8 and −9 expression are up-regulated.[25,26] In this regard, MMP-8 can proteolytically fragment the insulin receptor and, hence, may contribute to the development of diabetes.[27] MMPs also participate in important functions during gestation including trophoblast invasion, embryo implantation, and decidual development, and have been implicated in the pathophysiology of recurrent pregnancy loss and preterm prelabour rupture of membranes.[28-30] Within the past few years, we have been studying oral fluids, and specifically gingival crevicular fluid (GCF), as a suitable source for

potential biomarkers for early prediction of pregnancy diseases, because it seems concentrates placental derived molecules which could be collected in a simple, convenient, minimally-invasive, and optimal manner by a so-called chair-side point-of-care technology.[31-34]

This study aimed to establish the correlation between first trimester concentrations of MMP-8 and MMP-9 in GCF and the severity of the periodontal clinical inflammatory status, and the association with the subsequent development of GDM.

2 | MATERIAL AND METHODS

2.1 | Study design

A prospective cohort study was conducted between January 2017 and January 2019. The total cohort was composed of 314 pregnant women enrolled at 11 to 14 weeks of gestation. Each pregnant woman had prenatal care and was followed from enrollment until delivery. Demographic, clinical, physical, obstetric, and medical history was recorded. A single dentist with periodontology training performed a complete periodontal evaluation, including periodontal probing depth (PPD); clinical attachment level (CAL); bleeding on probing (BOP); plaque index (PI); (visible plaque accumulation), measured along the gingival margin and recorded as presence (+) or absence (−),[35] and periodontal inflamed surface area (PISA); PISA reflects the surface area of bleeding pocket epithelium in square millimeters and a freely downloadable spreadsheet is available to calculate the PISA (www.parsprototo.info).[36] This method quantifies the surface amount (mm^2) of inflamed periodontal tissue representing the inflammatory and infectious burden resulting from periodontitis.[36] All women were diagnosed according to their periodontal status. Pregnant women were enrolled at a single primary care center in Santiago, Chile. The diagnosis of GDM by oral glucose tolerance test (OGTT) at 24 to 28 weeks of gestation was used as the outcome variable. Participants who were diagnosed with GDM were assigned to the GDM group. Otherwise, they were assigned to the no-GDM group. Pregnant women were included if they have between 18 and 40 years old, with an 11 to 14 weeks pregnancy, with a prior pregnancy diagnosis confirmed by a transvaginal ultrasound scan, to confirm gestational age, number of embryos, and viability, with or without a diagnosis of periodontal disease. Women were excluded if they had: Type 1 or 2 diabetes, fewer than 18 teeth, with any history of periodontal therapy during the last 6 months, systemic or topical anti-microbial/anti-inflammatory therapy in previous 3 months. Written informed consent was obtained from women who agreed to participate in the study, that was approved by the Universidad de Los Andes Ethics Committee and was conducted in accordance with the Helsinki Declaration of 1973, as revised in 2003. All women with periodontal disease will receive case-specific treatment among the second trimester of pregnancy. The study protocol was clearly explained to all the participants, who signed informed consent.

The variables studied were GDM diagnosis, periodontal diagnosis, periodontal clinical measures (PPD, CAL, BOP, PI, PISA, and number of teeth), fasting first and second-trimester blood glucose concentration, age, first trimester body mass index (BMI), smoking during pregnancy, pre-pregnancy smoking history, and MMP-8 and MMP-9 in GCF concentrations during 11 to 14 weeks pregnancy.

2.2 | Definitions

GDM was diagnosed at 24 to 28 weeks of gestation using the following parameters: a criteria of diagnosis of GDM was made by 75 g oral glucose tolerance testing (OGTT) and 2 hour 8.5 mmol/L by universal glucose tolerance testing (fasting plasma glucose concentration of ≥93 mmol/L and a 2 hours blood glucose concentration of ≥153 mmol/L 2 hours after oral administration of 75 g glucose according to the IADPSG criteria).[2,3] Periodontitis was defined as stated by the 2017 World Workshop, as interdental CAL is detectable at ≥2 non-adjacent teeth, or buccal or oral CAL ≥ 3 mm with pocketing >3 mm is detectable at ≥2 teeth. Gingivitis was defined as subjects who did not exhibit PPD greater or equal to 3 mm, without CAL and positive BOP in ≥10%. Gingival health was defined as <10% bleeding on probing sites, with PPD ≤ 3 mm.[37,38] Staging for Periodontitis I and II was made based on the level of CAL. When CAL was between 1 to 2 mm, the diagnosis was Periodontitis Stage I. When CAL was between 3 and 4 mm, the diagnosis was Periodontitis stage II. When CAL was 5 mm or more, with 4 teeth or less missing and in presence or ten or more occluding pairs, in absence of bite collapse, drifting, flaring, or a severe ridge defect, the diagnosis was Periodontitis Stage III. In the presence of CAL more than 5 mm, with four or more missing teeth, absence of 10 occluding pairs, or when was observed bite collapse, drifting, flaring, or a severe ridge defect, the diagnosis was Periodontitis Stage IV.[37] The radiographic bone loss was not assessed because of the pregnancy condition. Therefore, the grade of periodontitis was not established.

2.3 | Sample size calculation

This is a secondary study from a principal cohort study, with a main objective related to preeclampsia (not

published yet). The estimated sample size was 358 pregnant women in their first trimester of pregnancy. The principal outcome of the prospective cohort study was preeclampsia (PE) development and its association with placental alkaline phosphatase (PLAP) in GCF in PE pregnancies, considering a PLAP mean of 2044.21 and 1880.56 pg/mL and standard deviation of 217.61 and 82.26 by PE and healthy pregnant women, respectively.[32] We considered a PE prevalence of 6.4%, a significance level of 5%, a power of 80%, for a two-sided test, and a loss of 10%.

2.4 | Gingival crevicular fluid (GCF) collection and elution protocol

GCF samples were obtained from four periodontal pockets and/or sites depending on periodontal diagnosis (most affected periodontal site/pocket × quadrant), at the same gestational age period in all pregnancies (11 to 14 weeks of gestation). The papers strips[*] were placed into the sulci/pocket until mild resistance was sensed and left in place for the 30 seconds and pooled. Strips contaminated by saliva or blood were excluded from the study. The collected GCF was subsequently eluted with 160 µL of elution buffer added 0.5 M Tris-HCl, pH 7.5, 2 M NaCl, 250 mM $CaCl_2$, and Triton-X100 at 25% of concentration, adding an EDTA-free protease inhibitor cocktail[†]; then, they were vortexed for the 30 seconds, incubated 30 minutes on ice, and centrifuged at 4°C for 5 minutes at 12,000 × g. The supernatant fluids were recovered and kept on ice. Samples were transferred into a new 1.5 mL microcentrifuge tubes and the process was repeated. The final 320 µL of the eluted samples were stored at −80°C for further analysis.

2.5 | Luminex assay

MMP-8 and MMP-9 concentrations in maternal GCF[‡] samples were quantified using a designed custom kit for Multiplex Elisa Assays.[§] Samples were analyzed using a multiplex assay,[¶] according to manufacturer instructions. All samples were analyzed by duplicate. The re-suspended microsphere cocktail (50 µL) was added to each well of a 96-well black plate. GCF eluate (50 µL) was added to each well.

The plates were carefully covered with an aluminum foil plate sealer and incubated at room temperature for 2 hours in a horizontal orbital plate-agitator adjusted to 800 ± 50 rpm. Plates then were placed in a specially designed magnet plate holder for 1 minute and the liquid was discarded. Each well was washed with 100 µL of wash buffer for 1 minute in the magnet plate and then the liquid was discarded again. Biotin antibody cocktail (50 µL) was added to each well, the plate was sealed and incubated at room temperature in an agitator for 1 hours. Each well was then washed with wash buffer as previously described and diluted Streptavidin-PE (50 µL) was added. The plate was incubated for 30 minutes, as previously described. A new wash was performed, following by a re-suspension of the microspheres in 100 µL of wash and incubation for 2 minutes. Finally, samples were analyzed by multiplex Elisa assays.[#] The final concentration of the samples was calculated using a software.[‖]

2.6 | Statistical analysis

The normality of continuous quantitative variables was assessed by the Shapiro Wilk test. They were described through the median and 25th and 75th percentile. The differences between GDM and no-GDM groups were evaluated by the Mann Whitney test. Categorical variables were described by absolute and percentage frequencies and evaluated with the Pearson chi-square test. Risk ratios (RR) were estimated through a Generalized Linear Model (GLM) with the log-binomial function, *P*-value, 95% confidence interval (95% CI) for each of the variables versus GDM status. Continuous variables were incorporated into the model as standardized variables. A complete model was estimated with variables that presented *P*-values ≤0.15 and what according to the literature could explain the differences. A final model was estimated with the least amount of variables that explain the differences between groups. Correlation coefficients were determined using test. Data management and statistical analyses were performed using Stata software.[**]

3 | RESULTS

A total of 314 pregnant women were enrolled in this prospective cohort study. Of them, 14% (45/314)

[*] Periopaper (ProFlow, Amityville, NY).
[†] Complete, Mini, EDTA-free Protease Inhibitor Cocktail, *EASYpack* (Roche, Basel, Switzerland).
[‡] Conical tubes 1.5 mL, high-clarity, flat-top screw cap (BD-Falcon, Bedford, MA).
[§] Luminex Human Magnetic Assay (2-plex), analytes MMP-8, MMP-9 (R&D Systems, Minneapolis, MN). Specifications: #cat LXSAHM-02 and #Lot L124550.
[¶] MAGPIX Multiplexing Instrument (Luminex Corp, Austin, TX.)
[#] MAGPIX Multiplexing Instrument (Luminex Corp, Austin, TX).
[‖] MILLIPLEX Analyst 5.1 software (Merck, Darmstadt, Germany).
[**] StataCorp. 2015. Stata Statistical Software: Release 14.1 (StataCorp LP, College Station, TX).

TABLE 1 Clinical, demographic and periodontal description of pregnant women at 11 to 14 weeks of gestation, who subsequently developed or no GDM

Variable	No-GDM ($n = 269$)	GDM ($n = 45$)	*P*
Age (years)	28 (25–32)	30 (27–34)	0.075
Weight (kg)	69 (60–77)	72 (64–86)	**0.011***
Height (m)	1.56 (1.50–1.61)	1.56 (1.51–1.60)	0.661
BMI (kg/m^2)	27 (24.42–31)	29.60 (26.75–32.45)	**0.001***
Systolic blood pressure	104 (100–110)	106 (100–112)	0.129
Diastolic blood pressure	63 (60–68)	63 (60–70)	0.472
Fasting glucose concentration	86 (82–87)	92.5 (86–95)	**<0.001***
Glucose concentration (2 hours) (second trimester)	83 (83–83)	93 (93–153)	**<0.001***
OGTT	103 (103–103)	153 (137–153)	**<0.001***
Smoking	41 (15.2%)	13 (28.9%)	**0.033**[a]
Pre-pregnancy smoking history	99 (36.9%)	25 (55.6%)	**0.021**[a]
Plaque Index (% sites)	65 (39–92)	73 (55–90)	0.333
Bleeding on probing (% BOP)	51 (30–76)	69 (35–88)	0.082
Periodontal probing depth (mean PPD)	2.6 (2.2–2.8)	2.8 (2.2–3.1)	0.068
Clinical attachment level (mean CAL)	2.0 (1.7–2.5)	2.2 (1.8–2.7)	0.244
Number of teeth	27 (25–28)	27 (25–28)	0.78
Periodontal inflamed surface area (mean PISA^{mm2})	707.8 (357.7–1159.6)	827.8 (475.4–1222.2)	0.299
Periodontal diagnosis			0.906
Gingival healthy	11 (4.5%)	2 (4.8%)	
Gingivitis	24 (9.7%)	2 (4.8%)	
Stage 1 periodontitis	21 (8.5%)	3 (7.1%)	
Stage 2 periodontitis	78 (31.6%)	14 (33.3%)	
Stage 3 and 4 periodontitis	113 (45.7%)	21 (50.0%)	

Abbreviations: GDM, gestational diabetes mellitus; BMI, body mass index; OGTT, oral glucose tolerance test. ***(bold)** significative *P*-value (< 0.05, Mann-Whitney test).

[a](Fisher exact test). Results are expressed as median, percentile 25 and 75.

developed GDM. All physical, clinical, medical, and periodontal variables are presented in Table 1. Women who developed GDM showed statistically significant differences in first trimester fasting glucose concentrations (*P*-value < 0.001), smoking habit (*P*-value = 0.033), pre-pregnancy history of smoking (*P*-value = 0.021), weight (*P*-value = 0.011) and body mass index (*P*-value = 0.001) at 11 to 14 weeks of pregnancy. Regarding the periodontal diagnosis: 4.8% of patients with GDM were diagnosed as gingival healthy, 4.8% had gingivitis, 7.1% periodontitis stage I; 33.3% periodontitis stage II, and a 50% had periodontitis stages III and IV. No significant differences were observed concerning the periodontal diagnoses (*P*-value = 0.906). All clinical parameters were worse in pregnant women who subsequently developed GDM, although without reaching statistical significance (Table 1). Also, there was observed a positive correlation between first trimester fasting blood glucose levels with CAL (Spearman rho: 0.12; *P*-value = 0.0358).

Pregnancy women diagnosed with periodontitis stage III and IV showed increased concentrations of MMP-8 in GCF at 11 to 14 weeks of pregnancy compared to pregnant women with periodontitis stage I (median value of 99.31 ng/mL, IQR: 85.32 versus 71.95 ng/mL, IQR: 54.04, respectively) (Figure 1). Concerning the concentrations of MMP-9, we identify a significant increase in pregnancies with periodontitis stage III and IV compared to periodontitis stage I (median value of 262.4 ng/mL, IQR: 312.55 versus 114.1 ng/mL, IQR: 184.94, respectively) (Figure 1). Furthermore, our results suggest a positive correlation between concentrations of MMP-8 and MMP-9 in GCF at 11 to 14 weeks of pregnancy with clinical inflammatory periodontal parameters such as BOP, PI, PPD, CAL, and PISA in GDM and no-GDM pregnancies (Table 2).

In addition to this, women who subsequently developed GDM had increased concentrations of MMP-8 and MMP-9 in GCF compared to those without GDM (Figure 2, Table 3). The median concentrations of MMP-8 in GCF in patients that subsequently developed GDM

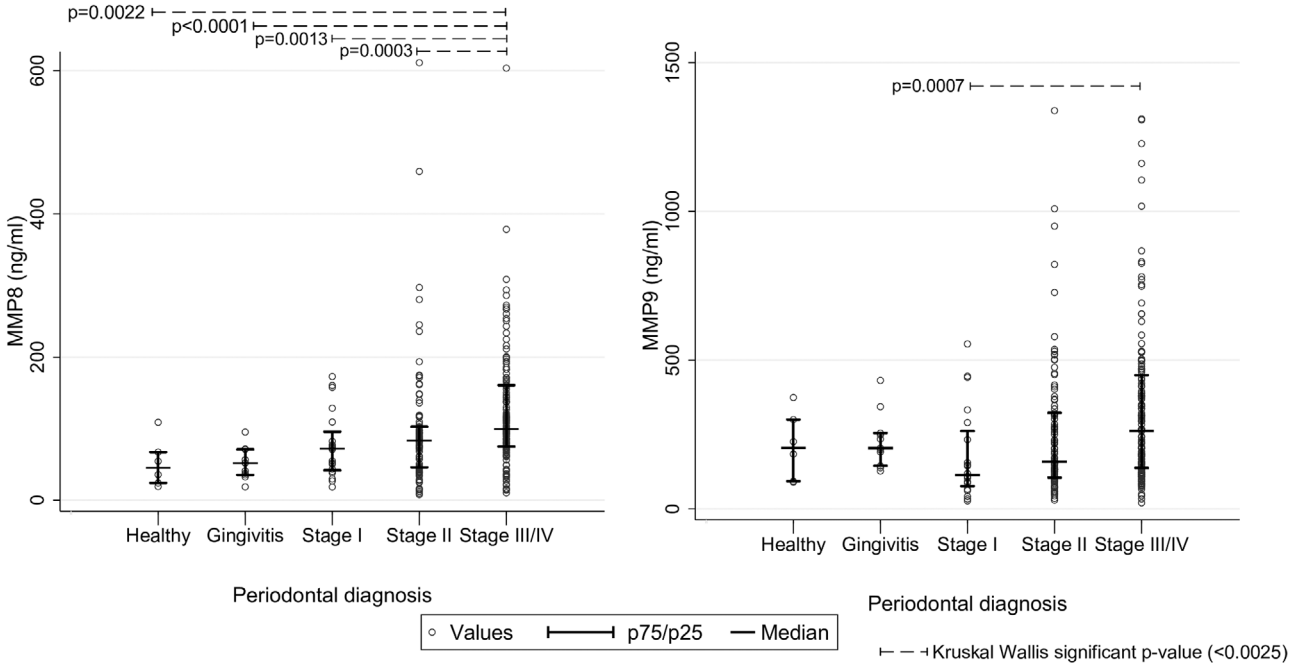

FIGURE 1 **A**) Concentrations of MMP-8 in gingival crevicular fluid at 11 to 14 weeks of gestation by periodontalclinical diagnosis. **B**) Concentrations of MMP-9 in gingival crevicular fluid at 11 to 14 weeks of gestation by periodontal clinical diagnosis

TABLE 2 Correlations between concentrations of MMP-8 and MMP-9 in GCF at 11 to 14 weeks of gestation and clinical periodontal parameters by GDM diagnosis

		BOP (%)	PI (%)	PPD (mm)	CAL (mm)	PISA (mm^2)
GCF MMPs		No-GDM GDM	No-GDM GDM	No-GDM GDM	No-GDM GDM	No-GDM GDM
MMP-8	*Rho*	0.44 0.34	0.33 0.38	0.43 0.31	0.34 0.41	0.47 0.20
	P-value	<0.0001* 0.0237*	<0.0001* 0.0108*	<0.0001* 0.0410*	<0.0001* 0.0058*	<0.0001* 0.1834
MMP-9	*Rho*	0.40 0.26	0.25 0.27	0.29 0.07	0.20 0.13	0.39 0.13
	P-value	<0.0001* 0.0801	0.0002* 0.0695	<0.0001* 0.6551	0.0030* 0.4019	<0.0001* 0.3758

Abbreviations: GDM, Gestational Diabetes Mellitus; GCF MMPs, gingival crevicular fluid metalloproteinase; MMP-8, metalloproteinase-8; MMP-9, metalloproteinase-9; BOP, bleeding on probing; PI, plaque index; PPD, periodontal probing depth; CAL, clinical attachment level; PISA, Periodontal inflamed surface area. (Rho Spearman's Test, * significative *P*-value < 0.05).

were 99.6 ng/mL (62.2 to 177.1) versus 86 ng/mL (52.4 to 118.3) in the no-GDM women (*P*-value = 0.0381). For MMP-9, median concentration in the GDM and no-GDM pregnancies were 243.8 ng/mL (157.2 to 433.9) and 183.6 ng/mL (110.6 to 374.1), respectively (*P*-value = 0.0302).

The unadjusted risk regression model suggested an association between the concentrations of MMP-8 during first trimester of pregnancy in GCF and the development of GDM (RR: 1.19; *P*-value = 0.045; CI 95% 1.00 to 1.40) (Table 4). Nevertheless, when the model was adjusted by age (years), BOP and BMI, the performance of the model decreases, but maintain a trend for the risk prediction of GDM (RR: 1.20; *P*-value = 0.063; CI 95% 0.99 to 1.45) (Table 4).

4 | DISCUSSION

The data of the present study suggest that GCF MMP-8 and MMP-9 concentrations, measured between 11 and 14 weeks of gestation are increased in pregnancies who develops GDM. In addition, fisrt trimester GCF MMP-8 concentrations could be associated with the subsequently development of GDM. Moreover, the increases of both MMPs were associated with the severity of periodontitis and also there were positively correlated with several periodontal clinical inflammatory parameters.

Several reports have shown that increased concentrations of MMP-8 in oral fluids are associated with periodontal inflammation and are correlated with progressive periodontitis disease activity.[23,24,39] Recently, a

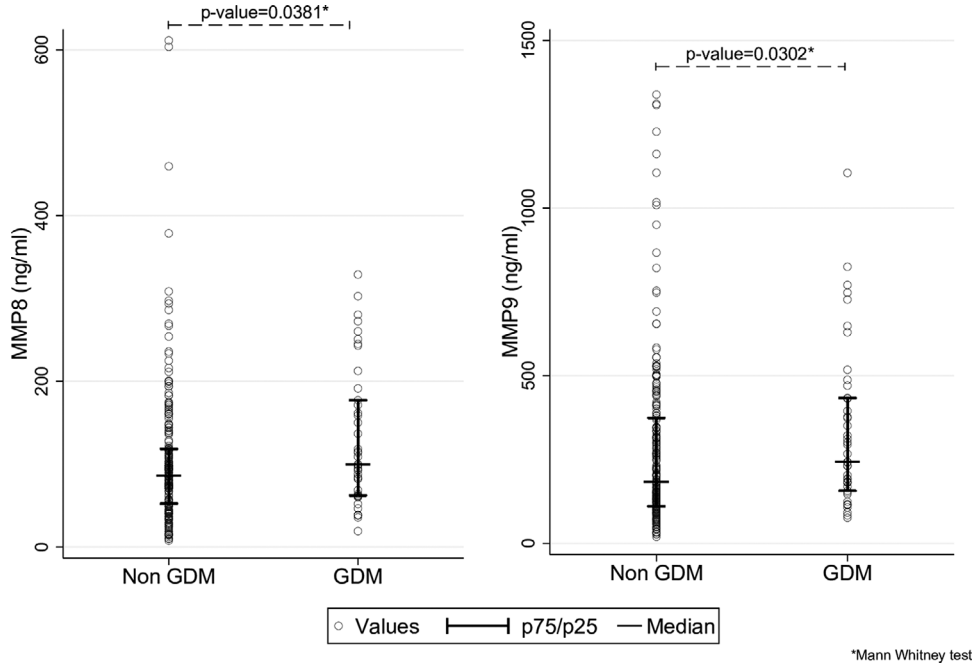

FIGURE 2 **A**) concentrations of MMP-8 in gingival crevicular fluid at 11 to 14 weeks of gestation in no-GDM and GDM pregnancies. **B**) Concentrations of MMP-9 in gingival crevicular fluid at 11 to 14 weeks of gestation in no-GDM and GDM pregnancies

TABLE 3 Description of concentrations (ng/mL) of MMP-8 and MMP-9 in GFC at 11 to 14 weeks of gestation by periodontal diagnosis and GDM status

GCF MMPs (ng/mL)		Gingival healthy	Gingivitis	Stage I periodontitis	Stage II	Stage III and IV periodontitis
MMP-8	*No-GDM*	45.2 (29.9–60.9)	46.7 (35.1–63.9)	72 (43.4–109.3)	82.4 (44.7–102.8)	96.3 (74.9–151.1)
	GDM	64.0 (19.2–108.9)	73.6 (51.9–95.3)	60.3 (38.1–82.4)	84.2 (60.1–92.9)	118.3 (97.9–177.1)
MMP-9	*No-GDM*	204.9 (136.8–262.9)	201.6 (141.0–251.2)	114 (65.6–289.9)	151.6 (99.5–317.2)	243.8 (129.7–430.5)
	GDM	233.4 (92.5–374.2)	311.6 (191.3–431.9)	127 (107–147.6)	231 (152.7–311.9)	243.8 (182.8–433.8)

Abbreviations: GDM, Gestational Diabetes Mellitus; GCF- MMPs, gingival crevicular fluid metalloproteinases concentration; MMP-8, metalloproteinase-8; MMP-9, metalloproteinase-9.

systematic review concluded that MMP-8 concentrations in oral fluids are up to 2.5-fold higher in patients with periodontitis when compared to healthy subjects.[40] In the periodontal tissue, MMP-8 and MMP-9 are released from neutrophils by supervised degranulation, triggered by potent periodontal bacteria together with host-derived proinflammatory mediators such as interleukin 1β and tumor necrosis factor-α.[24,39] At the same time, gingival fibroblasts stimulated by these cytokines can produce higher amounts of collagenolytic MMPs including MMP-8 and MMP-9, which are involved in the active destruction of periodontal tissues 23. In the present study, we have observed the same increase in concentrations of both MMPs in pregnant women from early pregnancy, and their concentrations were associated with periodontal clinical status, periodontitis severity stages and also with the subsequent development of GDM.

During pregnancy, there are several periodontal clinical changes (i.e., tenderness, redness, and gingival bleeding) related to an inflammatory state.[17-20] The systemic inflammatory state produced in pregnancy by the presence of the placenta could be related to the worsening of gingival/periodontal pre-pregnancy inflammation. We have demonstrated increased concentrations of placenta-derived molecules, such as placental growth factor, in GCF of pregnant women when compared with plasma, and further increase in prenatal diseases as GDM, even during early pregnancy.[33] This data supports the concept that placental derived molecules could regulate or at least modulate the periodontal inflammation process during pregnancy.

TABLE 4 Unadjusted and adjusted risk regression expressed as risk ratio (RR), *P*-value and confidence interval (95%) of the association between baseline variables at 11 to 14 weeks of gestation and GDM development (continuous variables are standardized)

Variables	Risk ratio	*P*	95% Confidence interval
Unadjusted Risk Regression Model			
MMP-8	1.19	**0.045***	1.00–1.39
MMP-9	1.15	0.216	0.92–1.42
Age (years)	1.38	**0.011***	1.08–1.78
BMI	1.33	**0.012***	1.06–1.66
Smoking history	1.53	0.159	0.84–2.78
Pregnancy smoking habit	1.44	0.200	0.82–2.49
Plaque index	1.16	0.315	0.87–1.53
Bleeding on probing	1.10	0.498	0.83–1.46
Periodontal probing depth	1.15	0.307	0.88–1.49
Clinical attachment level	1.18	0.167	0.93–1.49
Periodontal inflamed surface area	1.08	0.597	0.81–1.42
Adjusted Risk Regression Model			
MMP-8	1.20	0.063	0.99–1.45
Bleeding on probing	0.92	0.579	0.69–1.24
BMI	1.21	0.079	0.98–1.50
Age (years)	1.33	**0.033***	1.02–1.72

Abbreviations: GDM, Gestational Diabetes Mellitus; BMI, Body Mass Index; MMP-8 and MMP-9, Matrix Metalloproteinase-8.-9.***(bold)*** significantly *P*-value < 0.05, the results are expressed as risk ratio, *P*-value and confidence interval for each variable.

In the present study, we have shown that concentrations of MMP-8 and MMP-9 in GCF early in pregnancy are correlated with clinical signs of periodontal inflammation, and most importantly, are increased in women who will develop GDM later in pregnancy. To our knowledge, this study identifies for the first time increased concentrations of MMP-8 and MMP-9 in GCF during 11 to 14 weeks of gestation in women who later developed GDM.

A recent study has also demonstrated an increase in MMP-8 and MMP-9 GCF and serum concentrations in pregnant women with gingivitis and GDM in comparison with patients with GDM and healthy periodontium, suggesting that GDM could modulate both local and circulating concentrations of both MMPs, especially in pregnant patients with gingivitis.[41] However, this study was performed during the second trimester of gestation (24 to 28 weeks), at the time of GDM diagnosis, and also, the quantification method of MMPs was different to the current study. However, both studies suggest a tendency to have higher MMP-8 and MMP-9 concentrations in GCF of pregnant women with GDM and periodontal inflammation. MMP-8 can proteolytically fragment the insulin receptor, and this MMP-8-dependent insulin receptor proteolysis can be inhibited by MMP-8-inhibitors.[25,27] In this way, MMPs (especially higher concentrations of MMP-8) can contribute to the development of GDM.[26,27,42]

In the present study, we did not find an association between the periodontitis diagnosis and the subsequent GDM development. Probably, the young age of the pregnant women or the early recruiting time during pregnancy is possible explanations for this finding. However, all periodontal measures of inflammatory clinical parameters were worse in pregnant women who subsequently developed GDM, and this data is clinically relevant. Also, our results should be interpreted with caution, because the main outcome of this cohort study was the preeclampsia development, in which we report our preliminary results in GDM. These results require further prospective studies specifically aimed to analyze the association between GDM and periodontal disease. Moreover, considering that in Latino American population, the prevalence of the probing pocket depth ≥4 mm is 59.3% and severe periodontitis affects between 7.8% and 25.9% [43] of the population, it is possible than the association between GDM and periodontitis severity with the concentrations of MMP-8 and MMP-9 could be affected by the prevalence of the periodontitis in the studied population. Therefore, the real effect of the increase of MMP8 −9 in GDM pregnancies should investigate.

GDM and periodontitis may share common pathways such as a chronic inflammatory state and activity of pro-inflammatory mediators like MMPs activation, and that are also affected by confusing variables such as glucose regulation, obesity, smoking, and socioeconomic level. Studies investigating the association between periodontitis and GDM have demonstrated an elevated BMI associated with GDM 14, 15. In the present study, we also found and elevated BMI and smoking habit in GDM pregnancies, although both variables did not remain significant in the univariate and multivariate model, should be considered as variables that affect both periodontitis and GDM. Because of this, we required more specific studies with control of confounding variables, to analyze in-depth the association between periodontitis and GDM development during pregnancy. Besides, it is yet unknown whether the origin of MMPs in GCF aggravates the circulating concentrations of these enzymes, and how much of them come from the circulation or the inflamed periodontal tissues during pregnancy.

On the other hand,≈50% of women diagnosed with GDM are expected to develop type 2 diabetes and obesity over 10 to 30 years,[9-11] and also children of GDM mothers are at increased risk to become diabetic.[44,45] According to these arguments, coordinated efforts are required to provide better perinatal management and postpartum preventive strategies to modify these trends and to evade the

''vicious cycle'' in which diabetes causes more diabetes.[2,46] In this sense, countries with high rates of obesity are related to the highest rates of diabetes and our country is not an exception, in which the prevalence of GDM has increased from 4.4% in 2012 to 13% in 2015.[47]

GDM is diagnosed during the late second or early third trimester of pregnancy and at this time, the disease is already well established, and the possibility to reverse or limit potential adverse effects on perinatal outcomes is reduced. If an effective 1st-trimester screening test is available, the damage accumulated during the clinically occult phase (i.e., up to 24 to 28 weeks) could be potentially prevented by early interventions.[2,44,46] Nowadays, there are no tools available during early pregnancy to identify women who are at risk of developing GDM, and new approaches are required to meet this clinical need. Elevated oral fluid MMP-8 concentrations can be economically and conveniently analyzed by recent developed quantitative chairside point-of-care lateral flow immunotest resembling classical pregnancy test.[48,49] Therefore, early identification of oral biomarkers during the first trimester of pregnancy could represent a new approach to detect early the risk to develop GDM during pregnancy.

5 | CONCLUSIONS

Within the limitations of the present study, we suggest that first trimester MMP-8 and MMP-9 concentrations in GCF are increased in women who subsequently developed GDM and also are associated with periodontitis severity and correlated with the periodontal clinical inflammatory parameters. Their utility as early oral biomarkers for the onset GDM remains to be established and validated in larger prospective cohort studies.

AUTHOR CONTRIBUTIONS

All authors have made substantial contribution to conception and design of the study. Coceptualization: Alejandra Chaparro, Marcela Hernández, Timo Sorsa, Gregory Rice, and Sebastián Illanes. Data curation: Laura Weber, Fernanda Param, and Juan Pedro Kusanovic. Formal analysis: Valeria Ramírez and Daniela Albers. Funding acquisition: Alejandra Chaparro. Investigation: Alejandra Chaparro, Ornella Realini, Fernanda Param. Project administration: Marcela Hernandez, Ornella Realini, and Sebastián Illanes. Supervision: Timo Sorsa, Gregory Rice, and Juan Pedro Kusanovic. Writing original draft: Alejandra Chaparro, Marcela Hernández, Timo Sorsa, and Sebastián Illanes.

CONFLICT OF INTERESTS

The authors report no conflicts of interest, except Professor Timo Sorsa, who is an inventor of US-patent 10 488 415 B2 and a Japanese patent 2016-554676.

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