原　著

Detection of Periodontal Pathogens in Chorionic Tissues of Pregnant Women Diagnosed with Threatened Premature Labor and Abnormal Glucose Tolerance

Kozue Hasegawa-Nakamura*[1,2], Toshiaki Nakamura*[1,2], Masato Kamitomo*[3], Fumi Tateishi*[4], Chikoto Hashiguchi*[1,2], Kazuya Kawamata MD*[5], Masayuki Hatae*[6] and Kazuyuki Noguchi DDS, PhD*[1,2]

*[1]Department of Periodontology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

*[2]Research and Education Center for Advanced Oral Science, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

*[3]Department of Obstetrics and Gynecology, Kagoshima City Hospital, Kagoshima, Japan

*[4]Tadashige Dental Clinic, Tokyo, Japan

*[5]Women's Hospital Aiiku, Kagoshima, Japan

*[6]Kakinoki Hospital, Kagoshima, Japan

(Received: May 7, 2017　Accepted: June 12, 2017)

Abstract: The present study aimed to investigate the association between periodontal conditions and abnormal glucose tolerance (GT) in pregnant women with threatened premature labor (TPL). Fifteen normal pregnant women, 14 women with TPL and abnormal GT (TPL/abnormal GT), and 16 women with TPL and normal GT (TPL/normal GT) were enrolled. Periodontal parameters, including probing pocket depth and clinical attachment level, were examined. Detection of *Porphyromonas gingivalis* (*P. gingivalis*), *Fusobacterium nucleatum* (*F. nucleatum*), *Prevotella intermedia* (*P. intermedia*), *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, and *Tannerella forsythia* (*T. forsythia*), in saliva, subgingival plaque, and chorionic tissues from women was performed by using polymerase chain reaction. There were no significant differences in periodontal parameters and detection rates of periodontal pathogens from oral samples between women with TPL/normal GT and TPL/abnormal GT. *F. nucleatum*, *P. intermedia*, and *T. forsythia* were detected in chorionic tissues of women with TPL/abnormal GT, whereas *F. nucleatum* and *P. gingivalis* were detected in chorionic tissues of those with TPL/normal GT. No periodontal pathogens were detected in the chorionic tissues of normal pregnant women. In conclusion, our data do not indicate that periodontal disease is a risk factor for abnormal GT in TPL. However, the presence of *F. nucleatum*, *P. intermedia*, and *T. forsythia* in chorionic tissues in women with TPL may be involved in abnormal GT.

Nihon Shishubyo Gakkai Kaishi (J Jpn Soc Periodontol) 59 (3): 133-143, 2017

Key words: periodontal pathogens, abnormal glucose tolerance, chorionic tissues, threatened premature labor, periodontal disease

Introduction

Periodontal disease is a chronic inflammatory disease caused by multiple periodontopathic bacteria including *Porphyromonas gingivalis* (*P. gingivalis*), *Fusobacterium nucleatum* (*F. nucleatum*), *Prevotella intermedia* (*P. intermedia*), *Aggregatibacter actinomy-*

Corresponding author: Kazuyuki Noguchi
Department of Periodontology, Kagoshima University Graduate School of Medical and Dental Sciences
8-35-1 Sakuragaoka, Kagoshima 890-8544, Japan
E−mail: kazuperi@dent.kagoshima-u.ac.jp
doi：10.2329/perio.59.133

cetemcomitans（*A. actinomycetemcomitans*），*Treponema denticola*（*T. denticola*），and *Tannerella forsythia*（*T. forsythia*），resulting in breakdown of the tooth-supporting apparatus and loss of teeth. Maternal periodontitis is modestly, but independently, associated with adverse pregnant outcomes[1, 2]. Many studies have reported an association between periodontal disease and preterm low birth weight since the first report by Offenbacher *et al.* in 1996[3]. We have previously shown that the periodontal conditions of Japanese pregnant women with a diagnosis of threatened premature labor (TPL) are worse than those of normal pregnant women[4]. Additionally, *P. gingivalis* and *F. nucleatum* are found in chorionic tissues of pregnant women who are hospitalized because of a high risk of pregnancy, such as TPL, placenta previa, and multiple pregnancies, but not in chorionic tissues of normal pregnant women[5, 6]. Pregnant women with TPL are generally treated with tocolytic agents to prevent premature uterine contractions. Ritodrine, one of the tocolytic agents for treatment of TPL, is a β2-adrenergic stimulant and inhibits myometrial contractions[7, 8]. Ritodrine can affect glucose homeostasis[9]. Therefore, ritodrine may cause hyperglycemia in some women with TPL[10]. Maternal hyperglycemia during pregnancy is associated with increased rates of hypertension, preeclampsia, cesarean delivery, diabetes later in life in the mother, macrosomia, neonatal hypoglycemia, and shoulder dystocia in fetuses[11, 12].

Gestational diabetes mellitus (GDM), which shows abnormal glucose tolerance during pregnancy, is defined as a type of diabetes mellitus (DM) that is first diagnosed during pregnancy[13]. Risk factors for development of GDM include obesity, previous GDM, and a family history of DM[14]. Recently, some reports have indicated that GDM is related to the maternal inflammatory environment and abnormality in the placenta[15-17]. The association between periodontal disease and GDM has been investigated for the past 10 years. Periodontal conditions of women with GDM are significantly worse than those of women without GDM[18, 19]. Moreover, some reports have demonstrated that the detection rate of periodontal pathogens in oral samples and levels of inflammatory mediators in gingival crevicular fluid of women with GDM are significantly higher than those of normal pregnant women[20, 21]. However, other reports have concluded that there is no significant association between periodontal disease and GDM[22, 23]. Dasanayake *et al.* reported that there was no significant difference in clinical periodontal status between women with GDM and those without GDM, even though levels of *T. forsythia* in the vagina and serum C-reactive protein (CRP) levels in women with GDM were significantly higher than those in women without GDM[24]. Therefore, the correlation between periodontal disease and GDM is controversial. Consequently, additional studies to prove the association between periodontal disease and abnormal glucose tolerance during pregnancy are required.

The present study aimed to investigate the association between periodontal conditions and abnormal glucose tolerance in women with TPL.

Materials and Methods

The study protocol was approved by the Human Ethical Committee of Kagoshima City Hospital (H19 No8) and the ethical standards of the Kagoshima University Medical and Dental Hospital Human Ethical Committee (No. 20-18).

Participants

Hospitalized pregnant women who were diagnosed with TPL in the Department of Obstetrics and Gynecology, Kagoshima City Hospital and normal pregnant women in the Women's Hospital Aiiku were selected. Blood glucose levels of women with TPL at fasting and 2 h after eating were measured. Women in whom glucose levels were greater than 120 mg/dL one or more times were diagnosed as having abnormal glucose tolerance by obstetric doctors. Exclusion criteria were a history of DM or GDM, use of corticosteroids and antimicrobials during pregnancy, periodontal therapy during pregnancy, viral infection, and autoimmune disease. Written informed consent was obtained from all of the subjects.

Oral examinations

Oral examinations were performed in the women during their second trimester by one periodontist (K. H-N). Periodontal parameters, including the plaque index (PlI)[25], gingival index (GI)[26], clinical attachment level (CAL), probing pocket depth (PPD), and bleeding on probing (BOP) were examined at four sites (mesial, distal, buccal, and lingual/ palatal) per tooth either at the teeth on the right side of the maxilla and left side of the mandible or at the teeth on the left side of the maxilla and right side of the mandible. The presence of two or more teeth showing PPD of ≥4 mm and

Table 1 Primer sequences used in PCR.

primer sequence (forward/reverse)		Size (bp)	accession number	references
Porphyromonas gingivalis 16s rRNA		404	L16492	28)
forward	5'-AGGCAGCTTGCCATACTGCG-3'			
reverse	5'-ACTGTTAGCAACTACCGATGT-3'			
Fusobacterium nucleatum 16s rRNA		407	AJ_133496	29)
forward	5'-AGAGTTTGATCCTGGCTCAG-3'			
reverse	5'-GTCATCGTGCACACAGAATTGCTG-3'			
Prevotella intermedia 16s rRNA		575	NR_026119	28)
forward	5'-TTTGTTGGGGAGTAAAGCGGG-3'			
reverse	5'-TCAACATCTCTGTATCCTGCGT-3'			
Aggregatibacter actinomycetemcomitans 16s rRNA		557	NR_029171	28)
forward	5'-ATGCCAAATTGACGTTAAAT-3'			
reverse	5'-AAACCCATCTCTGAGTTCTTCTTC-3'			
Treponema denticola 16s rRNA		316	NR_036899	28)
forward	5'-TAATACCGAATGTGCTCATTTACAT-3'			
reverse	5'-TCAAAGAAGCATTCCCTCTTCTTCTTA-3'			
Tannerella forsythia 16s rRNA		641	NR_040839	28)
forward	5'-GCGTATGTAACCTGCCCGCA-3'			
reverse	5'-TGCTTCAGTGTCAGTTATAACT-3'			

CAL of ≥3 mm at the same site was defined as periodontitis, based on modification of the definition previously described by Lopez *et al.*[27]. Patients who were not diagnosed with periodontitis and had BOP were diagnosed with gingivitis.

Bacterial examinations

Subgingival plaque samples were obtained by inserting a sterilized paper point for 15 s into pockets of sites with inflammatory signs, including gingival redness and swelling. Saliva samples were collected using Salivettes (Sarstedt, Nümbrecht, Germany) before a periodontal examination. Part of chorionic tissues obtained by obstetric doctors after delivery were immediately fixed in 4% paraformaldehyde and then embedded in paraffin. Total DNA was purified from subgingival plaque, saliva and chorionic tissue samples using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Polymerase chain reaction (PCR) was carried out using the Taq PCR Core Kit (Qiagen) to detect *P. gingivalis*, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, *T. denticola*, and *T. forsythia*, in the DNA samples. The primer sequences, GenBank accession numbers, and predicted sizes of the PCR products used for PCR detection of the bacteria are shown in Table 1[28, 29]. PCR amplification was performed by 40 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and extension at 72℃ for 1 min. The PCR products were separated by 2% agarose gel electrophoresis, stained with ethidium bromide, and visualized using an ultraviolet light transilluminator (ATTO Corporation, Tokyo, Japan).

Clinical and anthropometric parameters

At the time of the oral examination, each partici-

Table 2 Comparison of clinical and anthropometric parameters

	TPL		Normal Pregnancy	p-value in 3groups comparison
	abnormal GT	normal GT		
	(n=14)	(n=16)	(n=15)	
age	33.8 (3.7)	29.6 (6.1)	34.8(5.7)	P=0.0940
BMI before pregnancy	25.4 (6.7)#	19.3 (4.5)	20.6 (2.3)	P=0.0327
Blood pressure	110.5/65.7	100.9/56.2	106.4/63.7	P=0.0585/P=0.0300
	(11.1/9.6)	(9.4/8.7)	(8.7/16.4)	
Smoking before pregnancy	3/14	2/16	0/15	P=0.211
medicine				
ritodrine	14/14	16/16	0/15	P<0.001
insulin	11/14	0/16	0/15	p<0.001
birth experience	10/14	12/16	7/15	P=0.246
history of PB	3/10	5/12	1/7	P=0.461
history of LBW	2/10	4/12	0/7	P=0.268
Neonatal weight	2760.5 (538.6)#	2090.1 (625.9)*	3013.3 (293.9)	P<0.001
Gestational week at delivery	35w+4d (2w+4d)*	33w+5d (3w+1d)*	38w+1d (0w+3d)	P<0.001
PB	9/14*	14/16*	0/15	p<0.001
LBW	7/14*	12/16*	0/15	p<0.001
giant baby	1/14	0/16	0/15	p=0.311

*: p<0.05 vs normal pregnancy
#: p<0.05 vs TPL/normal GT

pant was interviewed regarding height and weight measured before pregnancy, birth experience, smoking habits, and a history of DM, GDM, preterm birth (PB; birth before 37 weeks of gestation), and low birth weight (LBW; delivery of newborn with birth weight ＜2,500 g). Blood pressure on the day of the oral examination, medicine taken during the pregnancy, gestational age at delivery, and weight of the newborns were recorded after delivery. The gestational age was determined by measuring the crown rump length of the fetus with ultrasonographic tomography at the first trimester.

Statistical analysis

Nonparametric analysis of variance (Kruskal-Wallis test) was used to detect statistically significant differences between the three groups. When the Kruskal-Wallis test was significant, the Steel-Dwass post hoc test was performed for multiple comparisons. Differences in the ratios between the three groups were analyzed by Fisher's exact test. When Fisher's exact test was significant, the chi-square test was applied to analyze the differences between two groups. Results were considered statistically significant at $P < 0.05$. Statistical analyses were performed using statistical software (BellCurve for Excel, Social Survey Research Information Co., Ltd., Tokyo, Japan). All results are expressed as mean ± SD or the ratio.

Results

Analysis of clinical and anthropometric parameters

Table 2 shows the clinical and anthropometric parameters in this study. Fifteen women with a normal pregnancy (normal pregnancy group) and 30 women with TPL were analyzed in this study. Women with TPL had intravenous infusion of ritodrine. Fourteen women with TPL were diagnosed with abnormal glucose tolerance (TPL/abnormal GT group). Sixteen women with TPL did not have abnormal glucose tolerance or any clinical signs of other obstetric diseases (TPL/normal GT group).

The body mass index (BMI) of women in the TPL/abnormal GT group was significantly higher than that of women in the TPL/normal GT group ($P=0.0325$). Blood glucose levels of 11 women in the TPL/abnormal GT group were controlled by injection of insulin, and three women in this group were treated with diet. Neonatal weight in the TPL/normal GT group was significantly lower than that in TPL/abnormal GT group or normal pregnancy group (TPL/normal GT vs TPL/abnormal GT groups, $P=0.0316$; TPL/normal GT vs normal pregnancy groups, $P < 0.001$). Gestational weeks at delivery in the TPL/abnormal GT and

Table 3 Comparison of oral conditions.

	TPL		Normal Pregnancy	p-value in 3groups comparison
	abnormal GT (n=14)	normal GT (n=16)	(n=15)	
Remaining –teeth	28.4 (1.7)	28.7 (1.7)	28.4 (1.1)	p=0.8325
Treated teeth (%)	44.0 (15.6)	32.6 (19.4)	38.2 (15.2)	p=0.4193
Caries teeth (%)	7.1 (9.0)	8.5 (11.3)*	1.2 (2.8)	p=0.0341
PPD (ave)	2.1 (0.3)*	2.0 (0.3)*	1.7 (0.2)	p=0.0017
PPD≧ 4 mm (%)	5.8 (6.8)*	5.1 (9.3)*	1.0 (3.4)	p=0.0123
CAL(ave)	1.2 (0.9)*	1.1 (1.0)*	0.2 (0.2)	p<0.0001
CAL≧3mm(%)	20.2 (21.5)*	21.1 (24.6)*	1.0 (1.4)	p<0.0001
BOP	29.7 (23.0)*	24.2 (30.9)	10.8 (18.9)	p=0.0155
PlI (ave)	0.7 (0.4)*	0.6 (0.5)	0.3 (0.4)	p=0.0190
GI (ave)	0.5 (0.4)*	0.3 (0.3)	0.1 (0.2)	p=0.0063
diagnosis of periodontal disease				p=0.027
periodontitis	6/14	4/16	0/15	
gingivitis	8/14	11/16	14/15	
healthy	0/14	1/16	1/15	

*: p<0.05 vs normal pregnancy

TPL/normal GT groups were significantly lower than those in the normal pregnancy group (TPL/abnormal GT vs normal pregnancy groups, P=0.0047; TPL/normal GT vs normal pregnancy groups; P＜0.001). The number of PBs and LBWs were nine and seven in 14 women in the TPL/abnormal GT group, and 14 and 12 in 16 women in the TPL/normal GT group, respectively. There were significant differences in the ratios of PB (P＜0.001) and LBW (P＜0.001) among the three groups. The ratios of PB and LBW in the TPL/abnormal GT or TPL/normal GT group were significantly higher than those in the normal pregnancy group (PB: TPL/abnormal GT vs normal pregnancy groups, P＜0.001; TPL/normal GT vs normal pregnancy groups, P＜0.001 and LBW: TPL/abnormal GT vs normal pregnancy groups, P=0.002; TPL/normal GT vs normal pregnancy groups, P＜0.001). One pregnant woman in the TPL/abnormal GT group delivered a giant baby (neonatal weight ≥4000 g).

Comparison of oral conditions among the three groups

Table 3 shows the oral conditions of the participants in this study. Mean PPD, the percentage of PPD ≥4 mm, CAL, and the percentage of CAL ≥3 mm in the TPL/abnormal GT and TPL/normal GT groups were significantly higher than those in the normal pregnancy group (TPL/abnormal GT vs normal pregnancy groups: PPD, P=0.009; percentage of PPD ≥4 mm, P=0.0131; CAL, P＜0.0001; percentage of CAL ≥3 mm, P=0.0011 and TPL/normal GT vs normal pregnancy groups: PPD, P=0.0042; percentage of PPD ≥4 mm, P=0.0316; CAL, P=0.0149; percentage of CAL ≥3 mm, P=0.0051). Mean BOP (P=0.0078), PlI (P=0.0175), and GI (P=0.0102) in the TPL/abnormal GT group were significantly higher than those in the normal pregnancy group. There were no significant differences in periodontal parameters between the TPL/abnormal GT and TPL/normal GT groups. The ratios of subjects with periodontitis in the TPL/abnormal GT and TPL/normal GT groups were 6/14 and 4/16, respectively. None of the normal pregnant women were diagnosed with periodontitis.

Detection of periodontal pathogens in subgingival plaque, saliva, and chorionic tissues.

Table 4 shows the incidence of bacterial detection in subgingival plaque and saliva. Periodontal pathogens were detected in some oral samples from women in the TPL/abnormal GT group, TPL/normal GT group, and normal pregnancy group. There was a significant difference in the detection ratio of *P. intermedia* in saliva among the three groups (P=0.013). The detection ratio of *P. intermedia* in saliva of women in the TPL/abnormal GT (P=0.0006) or TPL/normal GT group (P=0.021) was significantly higher than that of women in the normal pregnancy group.

The detection ratios of periodontal pathogens in chorionic tissues are shown in Table 5. *F. nucleatum, P. intermedia*, and *T. forsythia* were detected in three chorionic tissues of 14 women in the TPL/abnormal GT group. The ratios of chorionic tissues in which *P. gingivalis* and *F. nucleatum* was detected in the TPL/normal GT group were 2/16 and 2/16, respectively. Periodontal pathogens were not detected in any chorionic tissues from normal pregnant women. Periodon-

Table 4 comparison of detection of periodontal pathogens in oral samples.

	TPL		Normal Pregnancy	p-value in 3groups comparison
	abnormal GT (n=14)	normal GT (n=16)	(n=15)	
Subgingival plaque				
P.gingivalis	6/14	4/16	4/15	p=0.564
F.nucleatum	14/14	16/16	12/15	p=0.058
P.intermedia	6/14	5/16	2/15	p=0.230
A.actinomicetemcomitance	1/14	1/16	1/15	p=1.000
T.denticola	5/14	5/16	2/15	p=0.373
T.forsythia	10/14	8/16	5/15	p=0.125
Saliva				
P.gingivalis	5/14	7/16	2/15	p=0.160
F.nucleatum	11/14	13/16	4/15	p=0.085
P.intermedia	6/14*	5/16*	0/15	p=0.013
A.actinomicetemcomitance	0/14	1/16	0/15	p=1.000
T.denticola	1/14	2/16	0/15	p=0.633
T.forsythia	6/14	7/16	3/15	p=0.311

*: p<0.05 vs normal pregnancy

Table 5 Comparison of detection of periodontal pathogens in chorionic tissues.

	TPL		Normal Pregnancy	p-value in 3groups
	abnormal GT (n=14)	normal GT (n=16)	(n=15)	
P.gingivalis	0/14	2/16	0/15	p=0.319
F.nucleatum	3/14	2/16	0/15	p=0.188
P.intermedia	3/14	0/16	0/15	p=0.026
A.actinomicetemcomitance	0/14	0/16	0/15	-
T.denticola	0/14	0/16	0/15	-
T.forsythia	3/14	0/16	0/15	p=0.026
Chorionic tissues in which any periodontal pathogen was detected	6/14*	3/16	0/15	p=0.012

*: p<0.05 vs normal pregnancy

tal pathogens were detected in chorionic tissue in 6/14 women in the TPL/abnormal GT group and in 3/16 women in the TPL/normal GT group. There were significant differences in the detection ratios of *P. intermedia* (P=0.026) and *T. forsythia* (P=0.026), and for the ratio of any periodontal pathogen detected in chorionic tissue (P=0.012) among the three groups. The ratio of chorionic tissues in which any periodontal pathogen was detected in the TPL/abnormal GT group was significantly higher than that in the normal pregnancy group (P=0.004).

Table 6 shows the clinical profiles of nine women in whom periodontal pathogens were detected in chorionic tissues. One woman in the TPL/abnormal GT group in whom *P. intermedia*, *F. nucleatum*, and *T. forsythia* were detected in chorionic tissues delivered a giant baby.

Discussion

Periodontal conditions in women with GDM are worse than those in women without GDM[18, 19]. Gogeneni *et al*. reported that GDM was significantly associated with increased levels of *P. gingivalis, Filifactor alocis, and T. denticola* in saliva[20]. In the present study, we could not show any significant differences in periodontal conditions and detection rates of periodontal pathogens from oral samples between women in the TPL/normal GT and TPL/abnormal GT groups. In our study, as soon as women with TPL were diagnosed with abnormal GT, their blood glucose levels were controlled by medical treatment. Therefore, hyperglycemia in women in the TPL/abnormal GT group might not have been high enough to affect their

Table 6 Clinical profiles of the women in whom periodontal pathogens detected in chorionic tissues.

		the detection of periodontal patthogens in chorionic tissues				TB/PB	NBW/LBW	periodontal diagnosis
		P. gingivalis	*F. nucleatum*	*P. intermedia*	*T.forsythia*			
TPL/abnormal GT	1	-	+	-	-	TB	NBW	gingivitis
	2	-	-	-	+	TB	NBW	gingivitis
	3	-	+	-	+	PB	NBW	gingivitis
	4	-	-	+	-	PB	LBW	periodontitis
	5	-	+	+	+	TB	giant baby	gingivitis
	6	-	-	+	-	PB	NBW	gingivitis
TPL/normal GT								
	1	+	+	-	-	PB	NBW	gingivitis
	2	-	+	-	-	TB	NBW	gingivitis
	3	+	-	-	-	PB	LBW	peirodontitis

TB: Term birth
NBW: Normal birth weight

periodontal condition. Additionally, the periodontal condition of women with GDM reported by Gogeneni *et al*.[20] (mean BOP: 70.3% and mean PPD: 2.3 mm) was worse than that of women in the TPL/abnormal GT group in our study (mean BOP: 29.7% and mean PPD: 2.1 mm). These findings may explain why we did not find any significant differences in the detection ratios of periodontal pathogens from oral samples between the TPL/normal GT and TPL/abnormal GT groups. In the present study, the periodontal condition of women in the TPL/normal GT or TPL/abnormal GT group was significantly worse than that of women in the normal pregnancy group, which is consistent with our previous reports that worsening periodontal status is associated with TPL[4,6]. TPL with a poor periodontal condition might have masked an association between periodontal disease and abnormal GT in the present study. It has been reported that higher pre-pregnancy BMI is associated with a higher prevalence of GDM[30]. In the present study, the mean BMI before pregnancy in women in the TPL/abnormal GT group (25.4 ± 6.7) was significantly higher than that of women in the TPL/normal GT group (19.3 ± 4.5). This finding suggests that the risk of pathogenesis of abnormal glucose tolerance in this study might be associated with a higher BMI before pregnancy, but not periodontal disease. Further studies focusing on the severity of periodontal disease in women with GDM are required.

In this study, *F. nucleatum*, *P. intermedia*, and *T. forsythia* were detected in chorionic tissues of the TPL/abnormal GT group, whereas *F. nucleatum* and *P. gingivalis* were detected in chorionic tissues of the TPL/normal GT group. No periodontal pathogens were detected in chorionic tissues of normal pregnant women. Additionally, there were significant differences in the ratios of any periodontal pathogen detected in chorionic tissue among the three groups. The number of periodontal pathogens (*F. nucleatum, P. intermedia*, and *T. forsythia*) in chorionic tissue from women in the TPL/abnormal GT group tended to higher than that from women in the TPL/normal GT group. Women with GDM have higher levels of *T. forsythia* in the vagina compared with women without GDM, although *Campylobacter rectus*, *F. nucleatum, T. forsythia, P. gingivalis*, and *T. denticola* have been detected in the vagina and cervical secretions in pregnant women with/without GDM[24]. Miranda *et.al.* showed that there is an association between higher levels of *T. forsythia*, *F. nucleatum*, and *P. intermedia* in the oral cavity and poor glycemic control in patients with DM[31]. Therefore, *F. nucleatum, P. intermedia*, and *T. forsythia* might be involved in abnormal glucose tolerance. Feng *et al.* suggested that activation of the toll-like receptor (TLR) 4-Myd88-NFkB pathway is related to upregulation of insulin resistance and higher maternal hyperglycemia[32]. The TLR4/Myd88/NFκB pathway is thought to be one of pathways activated by periodontal pathogens[33]. Some reports have indicated that GDM is related to the maternal inflammatory environment and abnormality in the placenta[15-17]. Our results and previous findings suggest the following: (i) some types of periodontal pathogens are more likely to invade and stay in the intrauterine tissue of women with abnormal glucose tolerance; and (ii) inflammatory reactions that are enhanced by periodontal pathogens in chorionic tissue affect abnormal glucose tolerance. However, the number of chorionic tissues that were analyzed was small and there was a small number of women in whom periodontal pathogens were detected in this study. Additionally, only two parts of chorionic tissues could be analyzed. We

could not perform quantitative analysis of periodontal pathogens in chorionic tissues and histological analysis of chorionic tissues focusing on inflammatory reactions because of the limitation of sampling of chorionic tissues. To investigate the effect of periodontal pathogens in chorionic tissues on pregnancy, large-scale *in vivo* and *in vitro* studies are required.

There are two possible pathways through which periodontal pathogens translocate to chorionic tissues: (i) bacteria in the oral cavity migrate to the chorionic tissues as a result of homogenous spread; and (ii) bacteria in the vagina and cervix spread to the uterus. Han *et al.* reported that *F. nucleatum* translocates from the mother's mouth to the placenta and fetus, and causes acute inflammation and stillbirth[34]. Furthermore, intravenous injection of *F. nucleatum* and *P. gingivalis* to pregnant mice are transmitted to the placenta and cause adverse pregnancy outcomes[35, 36]. Bacteremia occurs after chewing and tooth brushing in individuals with gingival inflammation[37]. Some periodontal pathogens that are detected in atherosclerotic plaques might be derived from the oral cavity through bacteremia[38, 39]. Therefore, the source of periodontal pathogens that were detected in chorionic tissues in this study might have been the oral cavity. However, in a few subjects, periodontal pathogens that were detected in chorionic tissues were not detected in oral samples. Therefore, we could not exclude the possibility that bacteria in chorionic tissues came from the vagina and cervix because we could not analyze vaginal and cervical samples. Further studies focusing on the dissemination of periodontal pathogens in chorionic tissues are required.

In this study, neonatal weight in the TPL/normal GT group was significantly lower than that in the TPL/abnormal GT group or normal pregnancy group. Additionally, abnormal pregnant outcomes of the TPL/abnormal GT group tended to be lower compared with those of the TPL/normal GT group. There are several risk factors for PB and LBW, including age, race, smoking, alcohol, experience of delivery, and a history of PB and LBW[40, 41]. In this study, there were no significant differences in age, smoking, experience of delivery, and a history of PB and LBW among the three groups. However, the ratios of birth experience and a history of PB and LBW in the TPL/abnormal GT group tended to be lower than those in the TPL/normal GT group. These findings may be due to lower abnormal pregnant outcomes in the TPL/abnormal GT group.

Conclusion

Our data do not indicate that periodontal disease is one of the risk factors for abnormal glucose tolerance in TPL women. However, this study shows the following. (i) The species of periodontal pathogens detected in chorionic tissues are different between women with TPL with normal glucose tolerance and those with TPL without glucose tolerance. (ii) The number of periodontal pathogens (*F. nucleatum*, *P. intermedia*, and *T. forsythia*) in chorionic tissue from women with abnormal glucose tolerance tends to be higher than that from women without abnormal glucose tolerance. These results suggest that *F. nucleatum*, *P. intermedia*, and *T. forsythia* s in chorionic tissues in women with TPL may be involved in abnormal GT. To investigate the association between periodontal disease and abnormal glucose tolerance during pregnancy, further clinical, large-scale studies of pregnant women without obstetric diseases other than GDM are required.

Acknowledgement

This study was partly supported by a Grant-in-Aid (23792483 and 26463139) for Scientific Research from the Japan Society for the Promotion of Science.

The authors have no conflicts of interest directly relevant to the content of this article.

References

1) Ide M, Papapanou PN: Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes—systematic review. J Periodontol, 84: S181-194, 2013.
2) Gomes-Filho IS, Cruz SS, Rezende EJ, Dos Santos CA, Soledade KR, Magalhaes MA, de Azevedo AC, Trindade SC, Vianna MI, Passos Jde S, Cerqueira EM: Exposure measurement in the association between periodontal disease and prematurity/low birth weight. J Clin Periodontol, 34: 957-963, 2007.
3) Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, McKaig R, Beck J: Periodontal infection as a possible risk factor for preterm low birth weight. J Periodontol, 67: 1103-1113, 1996.
4) Hasegawa K, Furuichi Y, Shimotsu A, Nakamura M, Yoshinaga M, Kamitomo M, Hatae M, Maruyama I, Izumi Y: Associations between systemic status, periodontal status, serum cytokine levels, and delivery

outcomes in pregnant women with a diagnosis of threatened premature labor. J Periodontol, 74: 1764-1770, 2003.
5) Hasegawa-Nakamura K, Tateishi F, Nakamura T, Nakajima Y, Kawamata K, Douchi T, Hatae M, Noguchi K: The possible mechanism of preterm birth associated with periodontopathic *Porphyromonas gingivalis*. J Periodontal Res, 46: 497-504, 2011.
6) Tateishi F, Hasegawa-Nakamura K, Nakamura T, Oogai Y, Komatsuzawa H, Kawamata K, Douchi T, Hatae M, Noguchi K: Detection of *Fusobacterium nucleatu*m in chorionic tissues of high-risk pregnant women. J Clin Periodontol, 39: 417-424, 2012.
7) Caughey AB, Parer JT: Tocolysis with beta-adrenergic receptor agonists. Semin Perinatol, 25: 248-255, 2001.
8) Simhan HN, Caritis SN: Prevention of preterm delivery. N Engl J Med, 357: 477-487, 2007.
9) Tsuchiya H, Ushijima K, Fujiwara Y, Fujimura A, Koshimizu TA: Chronic ritodrine treatment induces refractoriness of glucose-lowering beta2 adrenoceptor signal in female mice. Regul Toxicol Pharmacol, 62: 561-567, 2012.
10) Seltzer HS: Drug-induced hypoglycemia. A review of 1418 cases. Endocrinol Metab Clin North Am, 18: 163-183, 1989.
11) Kampmann U, Madsen LR, Skajaa GO, Iversen DS, Moeller N, Ovesen P: Gestational diabetes: A clinical update. World J Diabetes, 6: 1065-1072, 2015.
12) Fetita LS, Sobngwi E, Serradas P, Calvo F, Gautier JF: Consequences of fetal exposure to maternal diabetes in offspring. J Clin Endocrinol Metab, 91: 3718-3724, 2006.
13) Rayburn WF: Diagnosis and classification of diabetes mellitus: highlights from the American Diabetes Association. J Reprod Med, 42: 585-586, 1997.
14) Reece EA, Leguizamon G, Wiznitzer A: Gestational diabetes: the need for a common ground. Lancet, 373: 1789-1797, 2009.
15) Wedekind L, Belkacemi L: Altered cytokine network in gestational diabetes mellitus affects maternal insulin and placental-fetal development. J Diabetes Complications, 30: 1393-1400, 2016.
16) Guillemette L, Lacroix M, Battista MC, Doyon M, Moreau J, Menard J, Ardilouze JL, Perron P, Hivert MF: TNFalpha dynamics during the oral glucose tolerance test vary according to the level of insulin resistance in pregnant women. J Clin Endocrinol Metab, 99: 1862-1869, 2014.
17) Lowe LP, Metzger BE, Lowe WL Jr, Dyer AR, McDade TW, McIntyre HD; Group HSCR: Inflammatory mediators and glucose in pregnancy: results from a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. J Clin Endocrinol Metab, 95: 5427-5434, 2010.
18) Chokwiriyachit A, Dasanayake AP, Suwannarong W, Hormdee D, Sumanonta G, Prasertchareonsuk W, Wara-Aswapati N, Combellick J, Pitiphat W: Periodontitis and gestational diabetes mellitus in non-smoking females. J Periodontol, 84: 857-862, 2013.
19) Gumus P, Ozcaka O, Ceyhan-Ozturk B, Akcali A, Lappin DF, Buduneli N: Evaluation of biochemical parameters and local and systemic levels of osteoactive and B-cell stimulatory factors in gestational diabetes in the presence or absence of gingivitis. J Periodontol, 86: 387-397, 2015.
20) Gogeneni H, Buduneli N, Ceyhan-Ozturk B, Gumus P, Akcali A, Zeller I, Renaud DE, Scott DA, Ozcaka O: Increased infection with key periodontal pathogens during gestational diabetes mellitus. J Clin Periodontol, 42: 506-512, 2015.
21) Ozcaka O, Ceyhan-Ozturk B, Gumus P, Akcali A, Nalbantsoy A, Buduneli N: Clinical periodontal status and inflammatory cytokines in gestational diabetes mellitus. Arch Oral Biol, 72: 87-91, 2016.
22) Bullon P, Jaramillo R, Santos-Garcia R, Rios-Santos V, Ramirez M, Fernandez-Palacin A, Fernandez-Riejos P: Relation of periodontitis and metabolic syndrome with gestational glucose metabolism disorder. J Periodontol, 85: e1-8, 2014.
23) Esteves Lima RP, Cyrino RM, de Carvalho Dutra B, Oliveira da Silveira J, Martins CC, Miranda Cota LO, Costa FO: Association Between Periodontitis and Gestational Diabetes Mellitus: Systematic Review and Meta-Analysis. J Periodontol, 87: 48-57, 2016.
24) Dasanayake AP, Chhun N, Tanner AC, Craig RG, Lee MJ, Moore AF, Norman RG: Periodontal pathogens and gestational diabetes mellitus. J Dent Res, 87: 328-333, 2008.
25) Silness J, Loe H: Periodontal Disease in Pregnancy. Ii. Correlation between Oral Hygiene and Periodontal Condtion. Acta Odontol Scand, 22: 121-135, 1964.
26) Loe H: The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol, 38 (Suppl): 610-616, 1967.
27) Lopez NJ, Smith PC, Gutierrez J: Higher risk of preterm birth and low birth weight in women with periodontal disease. J Dent Res, 81: 58-63, 2002.
28) Ashimoto A, Chen C, Bakker I, Slots J: Polymerase chain reaction detection of 8 putative periodontal

pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol Immunol, 11: 266-273, 1996.

29) Vickerman MM, Brossard KA, Funk DB, Jesionowski AM, Gill SR: Phylogenetic analysis of bacterial and archaeal species in symptomatic and asymptomatic endodontic infections. J Med Microbiol, 56: 110-118, 2007.

30) Wei YM, Yang HX, Zhu WW, Liu XY, Meng WY, Wang YQ, Shang LX, Cai ZY, Ji LP, Wang YF, Sun Y, Liu JX, Wei L, Sun YF, Zhang XY, Luo TX, Chen HX, Yu LJ: Risk of adverse pregnancy outcomes stratified for pre-pregnancy body mass index. J Matern Fetal Neonatal Med, 29: 2205-2209, 2016.

31) Miranda TS, Feres M, Retamal-Valdes B, Perez-Chaparro PJ, Maciel SS, Duarte PM: Influence of glycemic control on the levels of subgingival periodontal pathogens in patients with generalized chronic periodontitis and type 2 diabetes. J Appl Oral Sci, 25: 82-89, 2017.

32) Feng H, Su R, Song Y, Wang C, Lin L, Ma J, Yang H: Positive Correlation between Enhanced Expression of TLR4/MyD88/NF-kappaB with Insulin Resistance in Placentae of Gestational Diabetes Mellitus. PLoS One, 11: e0157185, 2016.

33) Song B, Zhang YL, Chen LJ, Zhou T, Huang WK, Zhou X, Shao LQ: The role of Toll-like receptors in periodontitis. Oral Dis, 23: 168-180, 2017.

34) Han YW, Fardini Y, Chen C, Iacampo KG, Peraino VA, Shamonki JM, Redline RW: Term stillbirth caused by oral *Fusobacterium nucleatum*. Obstet Gynecol, 115: 442-445, 2010.

35) Han YW, Redline RW, Li M, Yin L, Hill GB, McCormick TS: *Fusobacterium nucleatum* induces premature and term stillbirths in pregnant mice: implication of oral bacteria in preterm birth. Infect Immun, 72: 2272-2279, 2004.

36) Belanger M, Reyes L, von Deneen K, Reinhard MK, Progulske-Fox A, Brown MB: Colonization of maternal and fetal tissues by *Porphyromonas gingivalis* is strain-dependent in a rodent animal model. Am J Obstet Gynecol, 199: 86 e81-87, 2008.

37) Forner L, Larsen T, Kilian M, Holmstrup P: Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. J Clin Periodontol, 33: 401-407, 2006.

38) Gibson FC 3rd, Yumoto H, Takahashi Y, Chou HH, Genco CA: Innate immune signaling and *Porphyromonas gingivalis*-accelerated atherosclerosis. J Dent Res, 85: 106-121, 2006.

39) Range H, Labreuche J, Louedec L, Rondeau P, Planesse C, Sebbag U, Bourdon E, Michel JB, Bouchard P, Meilhac O: Periodontal bacteria in human carotid atherothrombosis as a potential trigger for neutrophil activation. Atherosclerosis, 236: 448-455, 2014.

40) Fedrick J, Anderson AB: Factors associated with spontaneous pre-term birth. Br J Obstet Gynaecol, 83: 342-350, 1976.

41) Shiono PH, Klebanoff MA: Ethnic differences in preterm and very preterm delivery. Am J Public Health, 76: 1317-1321, 1986.

糖代謝異常を有する切迫早産妊婦の絨毛膜組織での歯周病原細菌の検出

中村　梢*1,2，中村利明*1,2，上塘正人*3，立石ふみ*4，
橋口千琴*1,2，川俣和弥*5，波多江正紀*6，野口和行*1,2

*1 鹿児島大学大学院医歯学総合研究科歯周病学分野
*2 鹿児島大学大学院医歯学総合研究科口腔先端科学教育研究センター
*3 鹿児島市立病院産婦人科
*4 忠重歯科医院
*5 医療法人愛育会愛育病院
*6 医療法人聖成会柿木病院

（受付日：2017 年 5 月 7 日　受理日：2017 年 6 月 12 日）

要旨：本研究は切迫早産（TPL）妊婦の歯周組織状態と糖代謝異常の関連を調べることを目的とした。被験者は 15 名の正常妊娠妊婦，14 名の切迫早産で糖代謝異常の妊婦（TPL/糖代謝異常），16 名の切迫早産で糖代謝正常の妊婦（TPL/糖代謝正常）とした。全ての妊婦にプロービングポケット深さなどの歯周組織検査を実施した。被験者から採取した唾液，歯肉縁下プラーク，絨毛膜組織の *Porphyromonas gingivalis*（*P. gingivalis*），*Fusobacterium nucleatum*（*F. nucleatum*），*Prevotella intermedia*（*P. intermedia*），*Aggregatibacter actinomycetemcomitans*，*Treponema denticola*，*Tannerella forsythia*（*T. forsythia*）の検出は PCR 法で行った。歯周組織検査結果と口腔内の歯周病原細菌の検出率は，TPL/糖代謝異常と TPL/糖代謝正常の間に有意差はなかった。絨毛膜組織絨では TPL/糖代謝正常は *F. nucleatum* と *P. gingivalis* が検出されたのに対し，TPL/糖代謝異常は *F. nucleatum, P. intermedia*，*T. forsythia* が検出された。正常妊娠妊婦の絨毛膜組織はいずれの歯周病原細菌も検出されなかった。本研究は歯周病が切迫早産妊婦の糖代謝異常のリスク因子と示せなかった。しかし絨毛膜に存在する *F. nucleatum*，*P. intermedia*，*T. forsythia* が糖代謝異常と関連する可能性を示唆した。

日本歯周病学会会誌（日歯周誌）59（3）：133-143，2017

キーワード：歯周病原細菌，糖代謝異常，絨毛膜組織，切迫早産，歯周病

連絡先：野口和行
〒890-8544　鹿児島市桜ヶ丘 8-35-1
鹿児島大学大学院医歯学総合研究科歯周病学分野
E−mail：kazuperi@dent.kagoshima-u.ac.jp