






The relationship between the anti-*Porphyromonas gingivalis* immunoglobulin G subclass antibody and small for gestational age delivery: a longitudinal study in pregnant Japanese women

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Background: Maternal serum IgG antibody against *Porphyromonas gingivalis* is an indicator of both periodontitis and adverse pregnancy outcomes. This study aims to evaluate the anti-*P. gingivalis* IgG and IgG subclasses 1–4 in threatened preterm labour (TPL) patients and their association with small for gestational age (SGA). **Methods:** Serum, saliva and subgingival plaque samples were collected from 47 TPL patients compared with 48 healthy pregnant women. The amount of *P. gingivalis* was measured in saliva and plaque using real-time polymerase chain reaction. The serum anti-*P. gingivalis* IgG titre and anti-*P. gingivalis* subclasses IgG 1–4 concentration were measured using enzyme-linked immunosorbent assay. **Results:** The amount of anti-*P. gingivalis* IgG-1 was significantly lower in the TPL group than in the healthy group. Fourteen subjects delivered SGA infants in the TPL group. The pocket probing depth (PPD), clinical attachment loss, PPD ≥ 5 mm%, amount of *P. gingivalis* in plaque, anti-*P. gingivalis* IgG and anti-*P. gingivalis* IgG-4 were significantly higher in the TPL-SGA group than in the TPL-normal weight group. Moreover, logistic regression analysis revealed the detection frequency of *P. gingivalis* in plaque and placenta weight were significantly correlated with SGA in TPL. In the receiver operating characteristic curve analysis, an amount of *P. gingivalis* in plaque ≥ 86.45 copies showed a sensitivity of 0.786 and a specificity of 0.727 (AUC 0.792) for predicting SGA in TPL. **Conclusion:** Lower anti-*P. gingivalis* IgG-1 amounts are related to TPL, while higher anti-*P. gingivalis* IgG and IgG-4 are related with SGA in TPL. Further, greater colonisation of *P. gingivalis* in plaque might increase the risk of SGA and can be useful in prediction of SGA in TPL.

Key words: *Porphyromonas gingivalis*, immunoglobulin G subclasses antibody, threatened preterm labour, small for gestational age

INTRODUCTION

Periodontal disease is a chronic inflammatory oral disease induced by bacteria. Accumulating epidemiological evidence suggests a potential association between periodontal disease and adverse pregnancy outcomes (APOs), including preterm birth (PB), low birth weight (LBW), and small for gestational age (SGA)^{1–4}. SGA, which is defined as a weight below the 10th percentile for the gestational age, is an

important prognostic trait because it predicts susceptibility to hypoglycaemia, hypothermia, erythrocytosis, and future growth suppression^{5,6}. Poor fetal growth is the leading cause of prenatal and infant mortality, and is a major public health problem worldwide.

Porphyromonas gingivalis is known to be a key-stone anaerobe in the progression to periodontal disease. It is also associated with progression of APOs^{7,8}. As a product of the host response against microbial colonisation, serum anti-*P. gingivalis* immunoglobulin

(Ig) G reflects the destructive periodontal disease status. However, the underlying mechanism of how the IgG subclasses for *P. gingivalis* affect pregnancy outcomes remains unknown.

In our previous study, we reported that *P. gingivalis* periodontal infection and its antibody response to homologous peptides of beta 2 glycoprotein I might be associated with both threatened preterm labour (TPL) and PB⁴. TPL is the progression of uterine neck dilatation and shortening caused by regular uterine contractions before 37 weeks of pregnancy, which is a risk factor for PB, LBW and SGA^{9,10}. Therefore, anti-*P. gingivalis* IgG is considered to be useful not only as a diagnostic parameter of periodontal disease^{12,13}, but also as a potential biomarker for APOs^{4,14}.

Human IgG antibody has four subclasses (IgG-1, -2, -3 and -4), and the immune response to most antigens includes a mixture of all four subclasses¹⁵. However, the underlying mechanism of how these IgG subclasses for *P. gingivalis* affect pregnancy outcomes remains unknown.

In this longitudinal study, we evaluated the periodontal parameters; amount of *P. gingivalis* in subgingival plaque and saliva samples, serum anti-*P. gingivalis* IgG, and IgG subclasses in TPL patients; and subsequent SGA status after childbirth to determine the association of the amount of anti-*P. gingivalis* IgG and IgG subclasses with SGA infant delivery.

MATERIALS AND METHODS

Ethics statement

This study was conducted in full accordance with the World Medical Association Declaration of Helsinki principles. The study and consent procedures were approved by the Institution Ethics Committee of Tokyo Medical and Dental University (No. 498). All human tissues were collected from the Obstetrics and Gynecology Clinic at Tokyo Medical and Dental University following the guidelines issued by Tokyo Medical and Dental University; written consent was obtained from all subjects who participated in the research.

Subjects

A total of 95 subjects, between the ages of 23 and 44 years, were recruited in our previous study⁴. Forty-seven TPL patients who were hospitalised in the Obstetrics and Gynecology Clinic at Tokyo Medical and Dental University met the inclusion criteria. These criteria included the following: regular uterine contractions occurring at least once every 10 minutes during 30 minutes of monitoring without dilatation

and effacement of the cervix, cervical length of < 2 cm, and/or vaginal bleeding between 22 and 28 weeks of gestation. Forty-eight healthy pregnant women visited the Obstetrics and Gynecology Clinic at Tokyo Medical and Dental University for regular prenatal check-ups (H group). The subjects had a minimum of 15 teeth remaining, with no systemic diseases.

After delivery, the gestational age at birth, birth weight and placental weight were recorded. The subjects in the TPL group were divided into the TPL-SGA and TPL-normal weight (NW) groups. SGA was defined as a birth weight of less than the 10th percentile for the gestational age in the Japanese standard. NW was defined as a birth weight of more than the 10th percentile for the gestational age in the Japanese standard¹⁷. PB was defined as birth at a gestational age of less than 37 weeks and LBW as a birth weight of < 2,500 g.

Periodontal examination

All 95 pregnant women received a periodontal examination between 26–28 weeks of gestation. The periodontal parameters included the pocket probing depth (PPD), clinical attachment loss (CAL), and bleeding on probing (BOP), which were recorded by one periodontal specialist (S.K.) at all six sites for each tooth using a manual probe (PCP-UNC15, Hu-Friedy, Chicago, IL, USA).

Oral and serum sample collection

Subgingival plaque samples were collected via 30-second insertion of a sterile #40 paper point from the deepest pocket of the Ramfjord teeth¹⁸, which include a right upper molar, an upper incisor, a left upper molar, a right lower molar, a lower incisor and a left lower molar. When the representative tooth was missing, the next tooth was used instead. One millilitre of unstimulated saliva was obtained simultaneously. The samples (plaque and saliva) were collected in capped sterile glass tubes and then stored at –80 °C. At 28 weeks of gestation, peripheral blood samples were collected and centrifuged at 1,500 g for 10 minutes at 4 °C. Thereafter, the serum samples were collected and immediately stored at –20 °C.

Bacterial detection

The prepGEM bacteria kit (ZyGEM, Hamilton, New Zealand) was used to extract DNA from the subgingival plaque and saliva samples according to the manufacturer's instructions. The extracted DNA was then subjected to real-time polymerase chain reaction (PCR) via the TaqMan probe detection method to determine the presence of *P. gingivalis*. The primers and probes

were prepared as follows: forward, TAGCTTGC-TAAGGTCGATGG; reverse, CAAGTGTATGCGGTTT TAGT; and probe, TGCGTAACGCGTATGCAACTTGCC. *Porphyromonas gingivalis* strain ATCC 33277, used as a positive control, was cultured under appropriate culture conditions¹⁹. The bacterial cells were counted using a Petroff-Hausser counting chamber, and their DNA was extracted from 10^2 to 10^8 cells as the standard. For the amplification reaction, duplicate samples were routinely used. The assays that were performed in a total volume of 25 μ L contained the following: 12.5 μ L Premix Ex Taq, 0.5 μ L each forward and reverse primer (final concentration, 10 nM), 0.5 μ L TaqMan probe (final concentration, 10 nM), 1 μ L template DNA solution, and an appropriate volume of sterilised DNase- and RNase-free water. The PCR TaqMan Master Mix without DNA was used as a negative control. Amplification and detection were performed using a thermal cycler device (Real-Time System II; Takara-bio, Tokyo, Japan) at 95 °C for 30 seconds, 95 °C for 5 seconds (40 cycles), and 60 °C for 30 seconds.

Serum anti-*Porphyromonas gingivalis* IgG titre determination

The anti-*P. gingivalis* IgG antibody titre was determined using a previously described enzyme-linked immunosorbent assay (ELISA) method²⁰. A sonic extracted antigen of *P. gingivalis* strain ATCC 33277 was prepared using the method of Yano-Higuchi *et al.*¹⁹. Briefly, 96-well assay plates (SUMILON multi-well plate for ELISA; Sumitomo Bakelite, Tokyo, Japan) were coated with the sonicated antigen (10 μ g/mL) in carbonate buffer and incubated for 2 hours at 37 °C. After blocking with 2% bovine serum albumin (BSA) in carbonate buffer, the plates were washed three times with phosphate-buffered saline (PBS)-T (1 \times PBS, 0.05% Tween 20, pH 7.2). Serially diluted healthy human serum (2^5 – 2^{14} , 100 μ L per well) was added as the standard. The testing serum samples were added to the duplicate wells. The plates were incubated for 2 hours at 37 °C and then washed three times. Thereafter, 100 μ L per well of alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemical, St Louis, MO, USA) was added. The plates were then incubated for 1 hour at 37 °C, washed three times, and developed with phosphate substrate (Sigma-Aldrich, St Louis, MO, USA). The optical density (OD) at 405 nm for each well was measured using the Vmax Microplate Reader (Molecular Devices, Downingtown, PA, USA).

The antibody titre calculation was performed using the method of Ishikawa *et al.*²⁰. Absorbance was plotted against the dilution ratio and the logarithm of the

dilution ratio. The regression curve (obtained with a cubic polynomial expression) intersected at the line expressed by an OD of 0.8 was adopted as the antibody titre for a given sample. The antibody titre was expressed as \log_2 of the dilution.

Serum anti-*Porphyromonas gingivalis* IgG subclass concentration determination

Serum anti-*P. gingivalis* IgG-1, -2, -3 and -4 concentrations were determined using ELISA. Briefly, in the 96-well assay plates, the first two lines that acted as the standards were coated with human IgG-1, -2, -3 or -4 Kappa purified myeloma protein (Sigma-Aldrich, St Louis, MO, USA) with concentrations serially diluted from 50 to 0.195 μ g/mL; the rest of the wells were coated with the sonicated antigen (10 μ g/mL) in carbonate buffer. The plates were then incubated for 2 hours at 37 °C. After blocking with 2% BSA in carbonate buffer, the plates were washed three times with PBS-T (1 \times PBS, 0.05% Tween 20, pH 7.2). Serially diluted serum (2^5 to 2^{14} , 100 μ L per well) was added into the duplicate wells, and the plates were incubated for 2 hours at 37 °C. Following incubation, the plates were washed three times. Subsequently, 100 μ L per well of dilution buffer and serum samples (1:64 diluted) was added into the standard line and the rest of the plates. Following another 1 hour of incubation at 37 °C, the plates were washed three times and developed with 100 mL secondary antibody (1:10,000 diluted): monoclonal anti-human IgG-1 biotin antibody produced in mice (8c/6), IgG-2 (HP6012), IgG-3 (HP6050) and IgG-4 (HP6025; Sigma-Aldrich); thereafter, they were subsequently incubated for 2 hours at 37 °C. Biotin-conjugated horseradish peroxidase-streptavidin (1:1,000 diluted; Vector Laboratories, Burlingame, CA, USA) was added, and the plates were incubated for 2 hours at 37 °C and washed three times. TMB substrate was added, and the reaction was stopped with 1 M H_2SO_4 (Sigma-Aldrich, St Louis, MO, USA) after 30 minutes; the plates were then read at 450 nm using the Vmax Microplate Reader.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 21 (IBM, NY, USA). Initially, the data distribution was assessed using the Shapiro–Wilk test. Thereafter, unpaired *t*-tests were applied to analyse the statistical differences in age, birth week, birth weight and placental weight between the TPL and H groups. Mann–Whitney *U*-tests were applied to analyse the mean PPD, mean CAL, percentage of sites with a PPD of ≥ 5 mm, percentage of BOP-positive

Table 1 Subject characteristics in the TPL group and H group

	TPL group (n = 47)	H group (n = 48)	P-value
Demographic characteristics			
Age (years)	33.5 ± 4.8	32.8 ± 3.9	NS
Smoking history (%)	29.8 (14/47)	20.8 (10/48)	NS
Periodontal parameters			
Mean PPD (mm)	2.34	2.3	NS
CAL (mm)	2.4	2.4	NS
Sites with a PPD of ≥ 5 mm (%)	0	0	NS
BOP (%)	20.7	22.2	NS
Laboratory values			
<i>Porphyromonas gingivalis</i> . in the plaque (16s rDNA copies)	1.29	0	NS
<i>Porphyromonas gingivalis</i> . (+)% in the plaque	51.0 (24/47)	43.7 (21/48)	NS
<i>Porphyromonas gingivalis</i> . in the saliva (16s rDNA copies)	43.6	204	NS
<i>Porphyromonas gingivalis</i> . (+)% in the saliva	53.2 (25/47)	60.4 (29/48)	NS
Titre of anti- <i>Porphyromonas gingivalis</i> . IgG	7.1	6.5	NS
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG1	17.2*	23.5	0.04
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG2	2.7	2.8	NS
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG3	3.2	3.3	NS
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG4	0.4	0.5	NS
Serum hs-CRP (ng/mL)	1048	1326	NS
Pregnancy outcomes			
PB rate	27.7 (13/47)*	2.1 (1/48)	0.0004
LBW rate	29.8 (14/47)*	6.25 (3/48)	0.003
SGA rate (%)	29.8 (14/47)*	6.25 (3/48)	0.003
Birth week	37.9 ± 2.2*	39.4 ± 2.8	0.0003
Birth weight (g)	2914.7 ± 490.1*	3107.6 ± 419.1	0.0438
Placenta weight (g)	617.0 ± 136.8	596.7 ± 113.4	NS

Parametric continuous variables were tested using an unpaired *t*-test and are given as means ± standard deviations; non-parametric continuous variables were tested using the Mann–Whitney *U*-test and are given as medians.

BOP, bleeding on probing; CAL, clinical attachment loss; H, healthy; hs-CRP, high-sensitivity C-reactive protein; Ig, immunoglobulin; LBW, low birth weight; NS, not significant; PB, preterm birth; PPD, pocket probing depth; SGA, small for gestational age; TPL, threatened preterm labour.

*Significantly different from the H group ($P < 0.05$).

sites, and amount of *P. gingivalis* in the saliva and plaque samples, anti-*P. gingivalis* IgG, and IgG subclasses between the TPL and H groups. The amount of *P. gingivalis* in the saliva and plaque samples that did not demonstrate a normal distribution was categorised into a dichotomous variable. The chi-square test was performed to compare the smoking history; incidence of PB, LBW and SGA; and detection frequency of *P. gingivalis* in the saliva and plaque samples between the TPL and H groups. The above-mentioned tests were also applied between the TPL-SGA and TPL-NW groups. Potential risk factors for TPL-SGA, including the mean PPD, mean CAL, percentage of sites with a PPD of ≥ 5 mm, detection frequency of *P. gingivalis* in the plaque samples, serum titre of IgG against *P. gingivalis*, serum concentration of IgG-4, and placental weight, were selected and used in the logistic regression analysis. The variables were screened using the stepwise approach and then evaluated using a regression model. Odds ratios and 95% confidence intervals (CIs) were also calculated. Receiver operating characteristic (ROC) curve analysis was used for the prediction of cut-off values. The diagnostic value of *P. gingivalis* in the plaque samples was evaluated by calculating the sensitivity and

specificity. *P*-values of < 0.05 were considered statistically significant.

RESULTS

TPL and H groups before delivery

Table 1 shows the demographic characteristics, periodontal parameters, laboratory values, and final birth outcomes of the subjects in the TPL and H groups. Although no significant difference was found in the demographic characteristics and periodontal parameters between the TPL and H groups, the serum anti-*P. gingivalis* IgG-1 concentration was significantly lower in the former than in the latter. After labour, 17 (17.9%) patients delivered SGA infants, and 78 (82.1%) delivered NW infants. Among the 17 subjects who delivered SGA infants, 14 and 3 were from the TPL and H groups, respectively. Among the 78 subjects who delivered NW infants, 33 and 45 were from the TPL and H groups, respectively. The birth week and birth weight in the TPL group were significantly lower than those in the H group. The incidence of PB, LBW and SGA was significantly higher in the TPL group than in the H group.

Table 2 Subject characteristics in TPL-SGA and TPL-NW groups

	TPL (47)		P-value
	TPL-SGA group (n = 14)	TPL-NW group (n = 33)	
Demographic characteristics			
Age (years)	35.1 ± 5.0	32.8 ± 4.6	NS
Smoking history (%)	42.8 (6/14)	24.2 (8/33)	NS
Periodontal parameters			
Mean PPD (mm)	2.6*	2.2	0.004
Mean CAL (mm)	2.6*	2.3	0.008
Sites with a PPD of ≥ 5 mm (%)	2.1*	0	0.019
BOP (%)	23.8	19	NS
Laboratory values			
<i>Porphyromonas gingivalis</i> . in the plaque (16s rDNA copies)	728*	0	0.002
<i>Porphyromonas gingivalis</i> . (+)% in the plaque	78.6 (11/14)*	39.3 (13/33)	0.014
<i>Porphyromonas gingivalis</i> . in the saliva (16s rDNA copies)	146	0	NS
<i>Porphyromonas gingivalis</i> . (+)% in the saliva	71.4 (10/14)	45.5 (15/33)	NS
Titre of anti- <i>Porphyromonas gingivalis</i> . IgG	8.6*	6.8	0.048
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG1	18.4	17.1	NS
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG2	3.1	2.3	NS
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG3	3.2	3.2	NS
Concentration of anti- <i>Porphyromonas gingivalis</i> . IgG4	0.9*	0.3	0.023
Serum hs-CRP (ng/mL)	1444	1408	NS
Pregnancy outcomes			
Birth week	36.7 ± 1.2*	38.4 ± 2.3	0.019
Birth weight (g)	2327.8 ± 114.4*	3153.1 ± 364.3	< 0.001
Placenta weight (g)	525.4 ± 92.9*	651.3 ± 135.8	0.005

Parametric continuous variables were tested using an unpaired *t*-test and are given as means ± standard deviations; non-parametric continuous variables were tested using the Mann–Whitney *U*-test and are given as medians.

BOP, bleeding on probing; CAL, clinical attachment loss; hs-CRP, high-sensitivity C-reactive protein; Ig, immunoglobulin; NS, not significant; NW, normal weight; PPD, pocket probing depth; SGA, small for gestational age; TPL, threatened preterm labour.

*Significantly different from the TPL-NW group ($P < 0.05$).

TPL-SGA and TPL-NW groups after delivery

In the TPL group, 14 patients delivered SGA infants (TPL-SGA group), and 33 delivered NW infants (TPL-NW group). *Table 2* shows the demographic characteristics, periodontal parameters, laboratory values, and pregnancy outcomes of the subjects in the TPL-SGA and TPL-NW groups. Regarding the periodontal parameters, the mean PPD and CAL and the percentage of sites with a PPD of ≥ 5 mm were significantly higher in the TPL-SGA group than in the TPL-NW group. Regarding the laboratory values, the anti-*P. gingivalis* antibody titre, *P. gingivalis* IgG-4 concentration, and amount and detection frequency of *P. gingivalis* in the subgingival plaque samples were significantly higher in the TPL-SGA group than in the TPL-NW group. The birth week, birth weight and placental weight in the TPL-SGA group were significantly lower than those in the TPL-NW group.

Potential risk factor of SGA in the TPL group

The logistic regression analysis showed that a high detection frequency of *P. gingivalis* in the plaque samples and the placental weight were significantly correlated with SGA in the TPL group (*Table 3*). The estimated odds ratios of *P. gingivalis* in the plaque samples (%) and the placental weight were 3.6 and 0.987, respectively.

Predictive factor of SGA in the TPL group

Figure 1 shows the ROC curve for the amount of *P. gingivalis* in the plaque samples for predicting SGA in the TPL patients. The area under the ROC curve for the amount of *P. gingivalis* in the plaque samples was 0.792 (95% CI, 0.634–0.951), and the cut-off value was 86.45 copies number, with a sensitivity of 0.786 and a specificity of 0.727.

DISCUSSION

The relationship between periodontal disease and pregnancy outcomes has been examined for the past 30 years. Previous studies have demonstrated that existing maternal periodontal disease might be a risk factor for APO^{2,21}. In the present study, we found increased severity of periodontal disease, including mean PPD, mean CAL and percentage of sites with a PPD of ≥ 5 mm, in the TPL-SGA group. These results correlate with previous findings. Boggess *et al.*³ reported an increased SGA rate among women with moderate or severe periodontal disease as compared with that among healthy women. Our data support the finding that severe periodontal conditions in pregnant women are also related to intrauterine growth restriction (IUGR), which is highly associated with neonatal mortality and life-long co-morbidities.

Table 3 Potential risk indicators for SGA in TPL subjects

	Estimated odds ratio	95% CI
<i>Porphyromonas gingivalis</i> . (+)% in the plaque	3.6*	1.2–22.8
Placenta weight	0.987*	0.977–0.998

CI, confidence interval.

*Statistically significant ($P < 0.05$; ordinal logistic regression analysis).

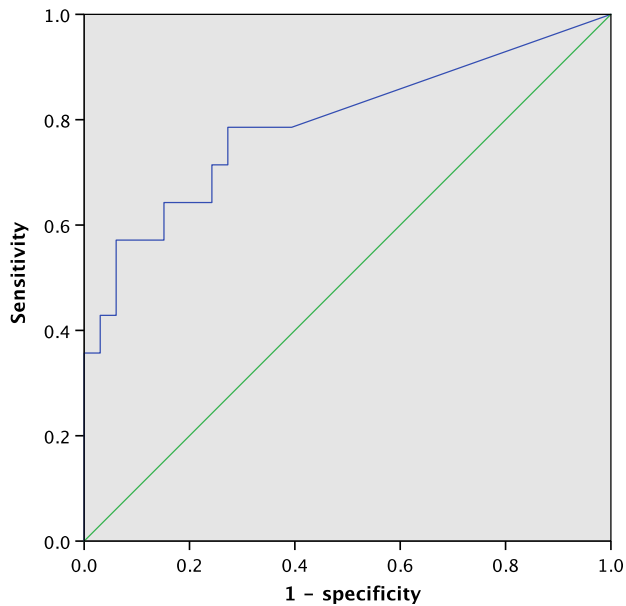


Figure 1 Receiver operating characteristic (ROC) curve for the amount of *Porphyromonas gingivalis* in the plaque samples for predicting small for gestational age (SGA) in threatened preterm labour (TPL). The area under the curve (AUC) for the amount of *P. gingivalis* in the plaque samples was 0.792 [95% confidence interval (CI), 0.634-0.951].

To the best of our knowledge, no study has yet evaluated the role of anti-*P. gingivalis* IgG subclasses in APOs. Only Sasahara *et al.*²² demonstrated that the anti-*P. gingivalis* IgG-1 concentration was lower in patients who had IUGR and PB than in healthy subjects. This might indicate the protective role of anti-*P. gingivalis* IgG-1 against APOs. Our study also demonstrated that the serum anti-*P. gingivalis* IgG-1 concentration in the TPL group was lower than that in the H group at the gestational age of 28 weeks.

Interestingly, the study findings correlate with those of Dasanayake *et al.*¹⁶ where an increment in the total IgG concentration in response to *P. gingivalis* in the mid-trimester was associated with the risk for LBW infant delivery. Conversely, Ebersole *et al.*²³ and Lin *et al.*¹⁴ have shown that women who delivered preterm infants had significantly lower total serum concentrations of IgG against *P. gingivalis* at the first trimester than women who delivered NW infants. Moreover, Madianos *et al.*²⁴ demonstrated that a lack

of maternal IgG against *P. gingivalis* within 48 hours after delivery was associated with an increased rate of premature birth. Different blood collection times and immunological alterations during pregnancy might contribute to these opposite conclusions. Hormones modulate the immunological shift that occurs during pregnancy²⁵. In early pregnancy, the maternal immune responses can control local and low-grade infections, and maternal IgG might protect the fetus from exposure to pathogens. As hormones change with progression of pregnancy, a shift from Th1 to Th2 immunity increases antibody production and pregnancy-related attenuation in immune responses. This will ultimately increase the susceptibility to and risk of developing complications of infections^{25,26}.

Optimal growth and function of the placenta are essential for fetal development. Lower placental weight not only affects the neonatal size but also results in a lower than expected body size late in childhood²⁷. Another risk factor in this study was the high detection frequency of *P. gingivalis* in the subgingival plaque samples. Animal studies revealed that after oral administration of *P. gingivalis*, a higher incidence of PB and LBW was found^{28,29}. Based on the results of the ROC analysis, the amount of *P. gingivalis* in subgingival plaques can be applied as a potential predictor to identify patients who are at a high risk of delivering SGA infants with TPL. Hence, periodontal therapy should be performed to reduce *P. gingivalis* colonisation in high-risk populations.

To the best of our knowledge, this is the first study to evaluate anti-*P. gingivalis* IgG subclasses in the serum and their possible relationship with APOs. Its limitations include the small number of patients included in the SGA group and its partial cross-sectional design, which makes it difficult to determine the changes in the IgG titres during pregnancy. Further investigation is necessary to reveal the mechanism and examine these findings in a larger population.

CONCLUSION

The present study demonstrated that low concentrations of anti-*P. gingivalis* IgG-1 were related to TPL, while high concentrations of total anti-*P. gingivalis* IgG and IgG-4 were related to SGA in TPL. The high prevalence of *P. gingivalis* in the plaque samples might be associated with an increased risk of SGA and might be useful in the prediction of SGA in TPL.

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Conflicts of interest

No conflicts of interest are reported for this study.

Author contributions

Hiroaki Kobayashi conceived and designed the research. Changchang Ye performed the experiments and wrote the manuscript with the help of Ryutaro Kuraji, Takeuchi, Yasuo, Hiroaki Kobayashi. Sayaka Katagiri performed the periodontal examinations. Naoyuki Miyasaka recruited subjects. Yuichi Izumi supervised the project. All authors reviewed the manuscript.

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