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Porphyromonas gingivalis, *Treponema denticola* and toll-like receptor 2 are associated with hypertensive disorders in placental tissue: a case–control study

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Aim(s): To explore the associations between the presence of periodontal pathogens and the expression of toll-like receptors (TLR-2 and TLR-4) in the placental tissue of patients with hypertensive disorders compared to the placentas of healthy normotensive patients.

Material and Methods: A case–control study was performed. From a cohort composed of 126 pregnant women, 33 normotensive healthy pregnant women were randomly selected, and 25 cases of patients with hypertensive disorders of pregnancy, including gestational hypertension and pre-eclampsia, were selected. Placental biopsy was obtained after aseptic placental collection at the time of delivery. All of the samples were processed and analysed for the detection of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola* and *Tannerella forsythia* using the polymerase chain reaction (PCR) technique. Determination of the expressions of TLR-2 and TLR-4 was performed in samples of total purified protein isolated from placental tissues and analysed by ELISA. The data were assessed using descriptive statistics. The associations among variables were estimated through multiple logistic regression models and the Mann–Whitney test to evaluate the differences between the two groups.

Results: A significant increase was observed in the expression of TLR-2 in the placentas of patients with hypertensive disorders ($p = 0.04$). Additionally, the multiple logistic regression models demonstrated an association between the presence of *T. denticola* and *P. gingivalis* in placental tissues and hypertensive disorders (OR: 9.39, $p = 0.001$, CI 95% 2.39–36.88 and OR: 7.59, $p = 0.019$, CI 95% 1.39–41.51, respectively).

Conclusions: In the present study, pregnant women with periodontal disease presented an association in the placental tissue between the presence of *T. denticola* and *P. gingivalis* and hypertensive disorders. Additionally, increased expression

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of TLR-2 was observed. However, further studies are required to determine the specific roles of periodontal pathogens and TLRs in the placental tissue of patients with pregnancy-related hypertensive disorders.

Periodontal diseases are among the most common chronic infections, affecting up to 50% of humans, and they have been found to be a putative independent risk factor for pregnancy-related complications, such as preterm births, low birth weight and hypertensive disorders of pregnancy, such as pre-eclampsia and gestational hypertension (1). Pregnant women with pre-eclampsia are predisposed to several potentially lethal complications, including placental abruption, disseminated intravascular coagulation, cerebral haemorrhage and hepatic and acute renal failure (2). The complications of pregnancy-related hypertensive disorders worldwide include maternal and foetal morbidity and mortality, as these complications cause nearly 40% of premature births before 35 wk of gestation. The pathogenesis of hypertensive disorders in pregnant women is not completely understood, but it is generally accepted that endothelial dysfunction of the maternal vascular system and infections play key roles in the pathogenesis of pre-eclampsia (3).

Periodontitis is a chronic destructive inflammatory pathology that is caused by a subgingival biofilm, in which gram-negative anaerobic microorganisms predominate. During prolonged periodontal inflammation, periodontal pathogens and related virulence factors enter the bloodstream by means of transient bacteraemia; these factors disseminate throughout different organ systems and affect the inflammatory response or directly colonize tissues (4,5). Furthermore, it has been proposed that the dissemination of bacterial endotoxins that originate from periodontal lesions might induce systemic activation of the inflammatory response (6,7).

Several bacterial species are found in subgingival plaque (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* and *Treponema denticola*). *Treponema denticola*, a gram-negative oral spirochete, has been shown to adhere to host cells and tissues, as well as to matrix proteins (8–12). These properties and this bacterium's high motility (13) could contribute to its possibly invasive capability and could be involved in the development of systemic non-oral diseases. *P. gingivalis*, another gram-negative periodontal bacterium, produces a broad array of potential virulence factors involved in tissue colonization and destruction, as well as in host defence activation. Both of these bacteria have been implicated as major aetiological agents in the development and progression of periodontitis (14,15). *F. nucleatum* is a gram-negative anaerobic species of the phylum fusobacteria. It is one of the most common oral species isolated from extra-oral infections, including blood, brain, chest, lung, liver, joint, abdominal, obstetric and gynaecological infections and abscesses. Further, *F. nucleatum* is a common anaerobic isolate from intrauterine infections and has been associated with pregnancy-related complications, including the delivery of premature low-birth-weight infants (16). *A. actinomycetemcomitans* is non-motile, facultative, anaerobic, small and gram-negative. Its fastidious, slow-growing nature makes it difficult to isolate through culture, especially because it is often a part of a mixed infection with other bacteria. *A. actinomycetemcomitans* has been found in various extra-oral infections, most frequently in endocarditis (17). The virulence mechanisms of *T. forsythia* are only just beginning to be defined, and the immune response to *T. forsythia* remains almost entirely undefined (18).

Toll-like receptors (TLRs) are transmembrane proteins that are evolutionarily conserved to recognize pathogen-associated molecular patterns in bacteria, fungi and parasites (19). In humans, 10 TLRs have been identified, the expression of all of which has been described in human placenta and gingival tissues. Individual TLRs recognize a particular surface or intracellular microbial molecule. The current evidence suggests that *P. gingivalis* is recognized primarily by TLR-2 (20,21).

Research on the expression of TLRs in the placenta has focused centrally on TLR-2 and TLR-4. The evidence suggests that the placenta recognizes microbes, promotes the innate immune response, increases cytokine responses and promotes immune cell migration (22). In general, TLR-2 and TLR-4 are constitutively expressed in term placentas from normal pregnancies (23); pre-eclampsia and other pathologies have been associated with increases in TLR-2 and TLR-4 in the placental tissue.

Holmlund *et al*. (24) first reported on the function of TLRs in the placenta. They demonstrated stimulation of TLR-2 and TLR-4 with zymosan and lipopolysaccharide-induced interleukin (IL)-6 and IL-8 production in third trimester placental cultures, which indicated that trophoblasts have the capacity to recognize microorganisms and to initiate immune responses by activating immune cells. Tinsley *et al*. (25) tested the effects of TLR activation on the development of pre-eclampsia-like symptoms in rats. These findings suggest that the activation of TLRs in pregnancy causes not only preterm labour and pregnancy loss but also pre-eclampsia. Gram-negative pathogens might play a role in the pathogenesis of hypertensive disorders. Indeed, infusion of low doses of *Escherichia coli* lipopolysaccharide into pregnant rats resulted in the activation of an inflammatory response and

subsequently in pre-eclampsia-like symptoms (26).

Several reports have indicated that periodontopathogens are recognized and that they activate host cells via TLRs. Studies have suggested that *T. denticola* and *P. gingivalis* activate the immune system via TLR-2 and that certain cytokines modulate this activation (27,28). Indeed, *T. denticola* was reported to induce cytokine production induced by cell-surface component recognition; for instance, peptidoglycan and lipopolysaccharides, both components of the outer membrane, induced the releases of IL-1β, IL-6, IL-8, metalloproteinase and prostaglandins (27). Furthermore, *T. denticola* induced IL-8 and monocyte chemoattractant protein-1 production in human umbilical cord endothelial cells (29).

The release of inflammatory cytokines further activates the inflammatory response, resulting in chronic low-grade systemic upregulation (30–32). It has been hypothesized that exacerbation of this inflammatory response during pregnancy by other pathologies might also be a predisposing factor for the occurrence of hypertensive disorders (33). Within this context, we previously demonstrated that the presence of periodontal disease was associated with an increase of IL-6 in the gingival crevicular fluid in early pregnancy and with the development of pre-eclampsia (34).

Our supposition was that pregnant patients with periodontal disease and hypertensive disorders had an increase in the haematogenous dissemination of periodontal bacteria to the placental tissue, stimulating TLR-2 and TLR-4 and contributing to the development of a local and systemic immune response.

The aim of the present study was to explore the association between the presence of periodontal pathogens and the expression of TLR 2 and 4 in the placental tissue of pregnant patients with periodontal disease and hypertensive disorders, compared to the placentas of healthy normotensive patients who also had periodontal disease.

Material and methods

Study design

A case–control study design was conducted. The selection criteria for cases were that they were pregnant women with diagnoses of hypertensive disorders, including pre-eclampsia and gestational hypertension. From a prenatal cohort of 126 women all of the cases ($n = 25$) were enrolled with diagnoses of hypertensive disorders. The control group was randomly selected using a randomization table from the same cohort, to generate a sample of 33 control subjects recruited from the obstetrics and foetal medicine unit of the University of the Andes Health Care Centre, Santiago, Chile. The sample size was arbitrarily established based on the number of pregnant patients enrolled with hypertensive disorders between March 2010 and December 2011. Women with any associated medical disorders (such as pre-existing hypertension, renal disease, anaemia, diabetes mellitus) were not considered for recruitment into this study. Both groups consisted of patients experiencing singleton gestation, who had the same socio-economic levels. The economic categorization used in this research took into consideration various characteristics of the pregnant woman's family, such as housing, belongings in the home and resident's educational status. These data were used to stratify the classification into good, regular or poor economic situation.

Women were excluded if they had fewer than 18 teeth, had undergone systemic or topical antimicrobial/anti-inflammatory therapy for the previous 3 mo or had a history of previous periodontal treatment. Dental evaluations and full-mouth periodontal exams were then performed by one periodontics, with a high intra-examiner reliability (0.806–0.812 kappa test). After their deliveries, all of the women with periodontal inflammation received case-specific treatment. Women who had teeth with urgent care needs were treated during pregnancy, and these women were excluded from the study. All clinically relevant data for the study were stored in a computer database. Written informed consent was obtained from the women who agreed to participate in the study, and the Universidad de los Andes Ethics Committee approved the study.

The variables studied were pre-eclampsia and gestational hypertension (pregnancy-related hypertensive disorders), age, initial weight, height, body mass index (BMI), tobacco use, and periodontal status. In placental tissue, the variables analysed were the detection of periodontal pathogens, including *P. gingivalis*, *F. nucleatum* spp., *T. denticola*, *T. forsythia* and *A. actinomycetemcomitans* and the expression of TLR-2 and TLR-4.

Definition of the study groups

The women were diagnosed with periodontitis if they had four or more teeth showing one or more sites with a probing pocket depth of 4 mm or greater, as well as clinical attachment loss of 3 mm or greater at the same site, inflammation and bleeding on probing (BOP). Women who showed BOP at more than 25% of sites and gingival redness but who did not have clinical attachment loss were diagnosed as having gingivitis. Pregnant women who did not exhibit probing pocket depth greater than or equal to 4 mm and attachment loss greater than or equal to 3 mm and fewer than 25% of the sites showed BOP were classified as normal (35).

The study groups consisted of patients with diagnoses of pre-eclampsia or gestational hypertension. Women were diagnosed with pre-eclampsia if, during the second and third trimester of pregnancy, they developed blood pressure over 140/90 mmHg and proteinuria, which was considered to be present when one 24 h urine collection showed a total protein excretion $\pm$ 300 mg. Gestational hypertension was defined in women if, during the second and third trimester of pregnancy, they developed a blood pressure over 140/90 mmHg without presenting proteinuria. The control group consisted of

women having normal pregnancies, defined as pregnancies that progressed without the development of any serious obstetric disease and that ended with the delivery of a healthy infant after 37 completed weeks of pregnancy and with normotensive blood pressure status (120/80 mmHg).

Clinical periodontal examination

All participants underwent a clinical periodontal examination. Each tooth was measured at six sites (mesiobuccal, buccal, distobuccal, lingual, distolingual and mesiolingual) with a North Caroline periodontal probe (Hu-Friedy, Chicago, IL, USA). Probing depth was recorded in millimeters from the gingival margin to the base of the gingival sulcus or periodontal pocket. Clinical attachment level measurements were determined using the cement–enamel junction as a reference point. BOP was measured (deemed positive if it occurred within 15 s after probing) and plaque index was scored as being present or absent at four sites per tooth (mesiobuccal, buccal, distobuccal and lingual surfaces).

Sample collection

Placental samples were taken from four central and peripheral sites on the maternal and foetal sides of the organ, the objective of the procedure was to obtain a representative sample of the placental tissue. Dissection was performed using a no. 15 blade and a standard test tube. Once obtained, the samples were numbered and coded, so the origin of the sample was masked for the remainder of the study. The samples were preserved in sterile Eppendorf tubes and stored at −80°C until further analysis. The sample collection was performed in the operating room using sterile methods.

Genomic DNA was obtained from the placental tissues using reagents from RBC Bioscience® [Genomic DNA extraction kit (tissue), catalogue no. YGT100, New Taipei, Taiwan]. All of the procedures were performed following instructions provided by the manufacturer. Then, the DNA obtained was used for conventional polymerase chain reaction (PCR) for periodontal bacterial detection.

The extracted protein was purified from the placental samples using reagents from Roche (Complete lysis-M, EDTA-free, catalogue no. 04719964001, Indianapolis, IN, USA) following the manufacturer's instructions. Protein quantification was performed using reagents from Thermo Scientific® (Pierce® BCA Protein Assay Kit catalogue no. 23225, Rockford, IL, USA). The proteins obtained were used for protein expression detection with ELISA.

Polymerase chain reaction

Genomic DNA was tested by conventional PCR. The presence or absence of five periodontal pathogens — *P. gingivalis*, *F. nucleatum* spp., *T. denticola*, *T. forsythia* and *A. actinomycetemcomitans* — was evaluated. The PCR reaction mixture was prepared with Promega® GoTaq® Green Master Mix (catalogue no. M7122, Madison, WI, USA), following the manufacturer's instructions. In the mixture was placed 5.2 μL of genomic DNA and 0.4 μL of each primer; the forward and reverse primers are shown in Table 1.

PCR analysis included an initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, an extension at 72°C for 45 s and a final extension at 72°C for 10 min. The amplified PCR product was run on 2% agarose gel. The photo gel was captured with an ENDURO™ GDS photo documentation system.

ELISA assays

The expression of TLR-2 and TLR-4 proteins was measured by ELISA using reagents provided by Abnova® (Human TLR2 Elisa Kit catalogue no. KA-1222 and human TLR4 Elisa Kit catalogue no. KA-1238, Taipei City,Taiwan), following the manufacturer's instructions. The ELISA plates were read at a wavelength of 450 nm in an automatic ELISA plate reader (Microplate Reader, ELx808, Biotek Instruments, Winooski, VT, USA).

Statistical analyses

Descriptive analysis of continuous variables (age and TLR expression) was performed based on the mean, standard deviation, median or interquartile range, depending on each variable. Dichotomous variables were tabulated with frequencies and percentages (bacterial presence, tobacco use and type of delivery). The differences among these variables in both groups (normotensive/hypertensive disorders) were evaluated using the Mann–Whitney test. The association strength was assessed using a multiple logistic regression model. The adjusted odds ratio (OR), by age, tobacco use and BMI with a 95% confidence interval (CI), was determined, and the data management and statistical analysis were performed using STATA software (version 11; StataCorp, Lakeway Drive College Station, TX, USA).

Table 1. Primers used for PCR analyses

Bacteria	Primer	Sequence
F. nucleatum	Fspp-1	5′-gga ttt att ggg cgt aaa gc-3′
	Fspp-2	5′-ggc att cct aca aat atc tac gaa-3′
T. denticola	Tden-1	5′-taa tac cga atg tgc tca ttt aca t-3′
	Tden-2	5′-tac aag aag cat tcc ctc ttc ttc tta-3′
T. forsythia	Tfor-1	5′-tac agg gga ata aaa tga gat acg-3′
	Tfor-2	5′-acg tca tcc cca cct tcc tc-3′
P. gingivalis	Pgin-1	5′-tgt aga tga ctg aaa acc-3′
	Pgin-2	5′-acg tca tcc cca cct tcc tc-3′
A. actinomycetemcomitans	Aa-1	5′-attg ggg ttt agc cct ggt g-3′
	Aa-2	5′-acg tca tcc cca cct tcc tc-3′

Results

Of the initial pregnant cohort of 126 patients, 25 developed hypertensive disorders, and 33 pregnant women served as random controls. Age, tobacco use, BMI and caesarean delivery data are shown in Table 2. There were no significant differences in socioeconomic level (low), age, weight, height or BMI between the groups ($p > 0.05$).

Both groups were similar regarding the number of patients with diagnoses of periodontal disease: periodontitis was found in 82% of patients who developed hypertensive disorders. In the control group, 77% of the patients were diagnosed with periodontitis. The mean probing depth and percentage of sites exhibiting BOP were similar between the groups, and no significant difference in the percentage of sites with plaque was found ($p > 0.05$, data not shown).

Several periopathogenic species were detected by PCR in the placental tissue samples of both groups. *T. forsythia* was present in a 21% of control group samples, compared to 40% in the hypertensive group. The presence of *A. actinomycetemcomitans* was 48% in the control group and 60% in the test group, and *F. nucleatum* was present in 73% of the control samples and 76% of the hypertensive group samples; results from both groups are presented in Table 3.

Multiple logistic regression analysis suggested an association between the presence of *T. denticola* (OR: 9.39; $p = 0.001$; CI 95% 2.39–36.88) and that of *P. gingivalis* (OR: 7.59; $p = 0.019$; CI 95% 1.39–41.51) in the placentas of pregnant women who developed hypertensive disorders during pregnancy (Table 4).

Additionally, increased expression of TLR-2 was observed in the placental tissue of women with hypertensive diagnoses ($p = 0.04$) (Fig. 1). The median value in the control group was 1.99 pg/mL, and it was 3.41 pg/mL in the hypertensive disorder group. TLR-4 was not expressed in 87.9% of control group samples and 80% of hypertensive patient samples. In both groups, only 20% of samples expressed TLR-4. The median value in the control group was 1.33 pg/mL ($n = 4$), and in the hypertensive disorder group, it was 3.48 pg/mL ($n = 5$). Nevertheless, there were no differences in the expression of TLR-4 between the two groups ($p = 0.32$).

Discussion

The present study showed an association between pregnant women with periodontal disease who were diagnosed with hypertensive disorders and the presence of *T. denticola* and *P. gingivalis* in their placental tissue. Additionally, increased expression of TLR-2 was found in the placental tissue of women with hypertensive disorder diagnoses.

Our results are in agreement with the outcomes observed by Barak *et al.* and Swati *et al.* (36,37), who established that periodontal pathogens are present in the placentas of women with pre-eclampsia and who reported significantly increased bacterial counts for all periopathogenic bacteria examined. A previous study evaluated the presence of periodontal pathogens in the subgingival plaque and amniotic fluid of 26 women with threatened premature labour, detected *P. gingivalis* in 30.8% of the patients (38).

Additionally, Swati *et al.* detected periodontal pathogens in the subgingival plaque and placenta of women with hypertension during pregnancy, and these periodontal pathogens were found to be more prevalent in the group with hypertension than in the controls. The authors suggested that the organisms might have been transmitted haematogenously and participated in the formation of atherosis in placental tissue and pre-eclampsia development; they proposed that the virulence of these bacteria could be responsible for hypertension during pregnancy (37).

Chronic periodontal inflammation might be a potential source of inflammatory molecules that can disseminate and act systemically in the vascular endothelium to promote endothelial lesions and thereby increase the risk of systemic pathologies (39–42). This association is supported by the finding that oral infections stimulate the immune inflammatory response via a local increase in proinflammatory mediators, such as IL-1β, prostaglandin E_2, IL-6 and tumour necrosis factor-α (8,9,42,43). Additionally, oral bacteria have been detected in atherosclerotic plaques, and they can play roles in the development and progression of atherosclerosis, leading to coronary vascular disease (44). It is known that the placental lesions in pre-eclampsia share a similar pathogenesis (inflammation) and clinical

Table 2. Characteristics of normotensive and hypertensive patients

Variables	Healthy/normotensive patients $n = 33$	Hypertensive disorder patients $n = 25$
Age	24.75 (6.2)*	28.32 (7.98)*
Smoking	6.06%	8%
Caesarean delivery	30.3%	48%
Body mass index	26.53 (6.5)*	26.31 (7.31)*

*Mean (SD).

Table 3. Presence of periodontal bacteria in both groups (normotensive and hypertensive patients)

Bacteria	% in placenta (normotensive)	% in placenta (hypertensive)
T. forsythia	21	40
A. actinomycetemcomitans	48	60
T. denticola	24	64
P. gingivalis	64	92
F. nucleatum	73	76

Table 4. Association between periodontal bacteria and hypertensive disorder status (multiple logistic regression, adjusted according to age, tobacco use and body mass index) *$p < 0.05$

Bacteria	Odds ratio	*p* Value*	95% CI
T. forsythia	2.37	0.167	0.70–8.01
A. actinomycetemcomitans	2.01	0.230	0.64–6.31
T. denticola	9.39	0.001*	2.39–36.88
P. gingivalis	7.59	0.019*	1.39–41.51
F. nucleatum	1.34	0.662	0.36–5.07

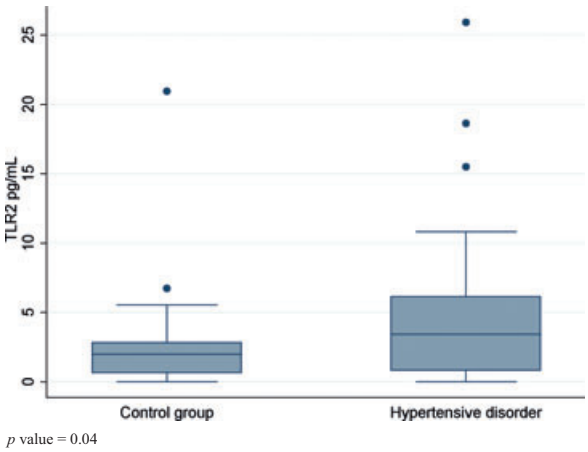

Fig. 1. Toll-like receptor (TLR)-2 expression in normotensive (control) and hypertensive groups. *p* value = 0.04

setting (endothelial cell damage) to those of atherosclerosis (5,6,44). Thus, it is reasonable to presume that periodontal pathogens could play potential pathogenic roles in the development of pregnancy-related hypertensive disorders.

Another possible option suggests that the hypertensive state may cause an increased vascular permeability of inflamed periodontal tissue and easier access for periodontal pathogens into the circulation and facilitates the translocation of oral bacteria or bacterial products to a maternal–foetal interface and cause an inflammatory immune response in placental tissue.

Indeed, our research group recently reported that pregnant women with periodontitis who later developed pre-eclampsia showed increased levels of IL-6 in gingival crevicular fluid and C-reactive protein in plasma during early pregnancy (34).

TLRs are pattern recognition receptors that play key roles in the innate inflammatory response, and they have been proposed to play important roles in pregnancy maintenance, placental immune protection and delivery initiation (45). Holmlund *et al.* were the first researchers to report on TLR function in placenta. They showed that the stimulation of TLR-2 and TLR-4 with zymosan and lipopolysaccharide induced the production of proinflammatory cytokines IL-6 and IL-8, which indicated that trophoblasts have the capacity to recognize microorganisms and initiate the immune response by activating immune cells (24).

Current evidence suggests that *P. gingivalis* might have developed ways to evade the TLR system, which senses this organism primarily through TLR-2 (20,21). These observations might be unexpected given that *P. gingivalis* is a gram-negative organism that expresses lipopolysaccharide. However, this bacterium is capable of modifying its structure, resulting in a biologically inert lipopolysaccharide molecule, which thereby allows *P. gingivalis* to evade TLR-4 activation (46).

Furthermore, *T. denticola* activates TLR-2, rather than TLR-4 (24,25,46). In fact, Nussbaum *et al.* reported that the absence of TLR-4 does not reduce the macrophage response to *T. denticola*, suggesting that TLR-2 activation is sufficient to achieve a maximal effect (27).

Hasegawa-Nakamura *et al.* examined the effects of *P. gingivalis* lipopolysaccharide on the production of proinflammatory molecules in cultured chorionic-derived cells. The authors concluded that the lipopolysaccharide of *P. gingivalis* induced IL-6 and IL-8 generation via TLR-2 in chorionic-derived cells at significantly higher levels than in control cultures (47).

These findings imply the possibility that the presence of *P. gingivalis* and *T. denticola* in chorionic tissues could affect pregnancy outcomes, and they are in agreement with our results, which indicate an increase in TLR-2 expression. It is probable that trophoblastic cells recognized *P. gingivalis* and *T. denticola*, via TLR-2 and subsequently induce the synthesis and release of proinflammatory cytokines.

There are two possible pathways through which periodontopathogens can translocate to chorionic tissues, e.g. (i) bacteria in the oral cavity migrate to the chorionic tissues as a result of haematogenous dissemination, and (ii) bacteria in the vagina and cervix spread to the uterus. Furthermore, *F. nucleatum* intravenously injected into pregnant mice was transmitted to the placenta and caused adverse pregnancy outcomes (48). In other studies (49–51) in which pregnant rodents were injected with *P. gingivalis*, foetal weight was decreased, whereas embryo mortality and the percentages of foetal resorption and foetal growth restriction increased. Exposure of sheep to lipopolysaccharides from *P. gingivalis, A. actinomycetemcomitans* and *F. nucleatum* caused much higher rates of foetal mortality than *E. coli* lipopolysaccharides did (52).

In the present study, the results suggest no difference in the presence and severity of periodontal disease in both groups. However, the diagnosis

of pregnant patients with hypertensive disorders presented an increased presence of *P. gingivalis* and *T. denticola*. Probably, the hypertensive state together with an increased production of female hormones and the extensive vascularity of the periodontium may cause an increased vascular permeability of periodontal tissues and easier access for periodontal bacterias to the circulation and placental tissues.

In this research, the placental tissues were obtained from caesarean section (37.93%) and vaginal delivery (62.07%), and although the dissection was performed under sterile conditions, the placentas obtained by vaginal delivery are likely to have been contaminated, which is a limitation of the present study that should be considered. Another drawback was the method of bacterial detection by conventional PCR; currently, the gold standard technique for bacterial detection is real time –PCR. Finally, it is important to note that this study is an association study and the cause and effects are not established in the present investigation.

In conclusion, the present study suggests that in this group of pregnant patients with periodontal disease, we observed an association between the presence of *P. gingivalis* and *T. denticola* in placental tissue and hypertensive disorders, as well as amplified expression of TLR-2. Deeper understanding of the immunology of the maternal foetal interface promises to yield significant insight into the pathogenesis of hypertensive disorders. We believe that further prospective longitudinal studies are needed to characterize placental TLR recognition in response to oral pathogens in placental tissues, to elucidate and validate the pathogenic mechanisms involved in hypertensive disorders and to assess the impact of periopathogenic bacteria in placental tissue of pregnant patients with periodontal diseases.

Conflict of interest

The authors declare that they have no conflicts of interest with regard to this study.

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