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REVIEW

The potential association between gingival crevicular fluid inflammatory mediators and adverse pregnancy outcomes: a systematic review

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Abstract
Objectives The association between periodontal disease and adverse pregnancy outcomes (APO), primarily preterm birth (PTB), is still controversially discussed in the literature. Therefore, the aim of the present systematic review was to analyze the existing literature on the potential association between inflammatory mediators detected in gingival crevicular fluid (GCF) and APO.
Materials and methods MEDLINE (PubMed) and EMBASE databases were searched for entries up to April 2012 and studies were selected by two independent reviewers.
Results The majority of the eight studies included confirmed a positive association between GCF mediators, such as interleukin-1β, prostaglandin E_2, and tumor necrosis factor-alpha, and APO. Due to the heterogeneity and variability of the available studies, no meta-analysis could be performed.
Conclusions A positive association between GCF inflammatory mediator levels and APO/PTB might be present but the results need to be considered with great caution because of the heterogeneity and variability among the studies. Further studies with an adequate number of patients allowing for an appropriate analysis are warranted to definitely confirm this association.
Clinical relevance The present findings suggest that an association between GCF inflammatory mediator levels and APO might exist.

Keywords Periodontal disease · Gingival crevicular fluid · Inflammatory mediators · Adverse pregnancy outcomes · Preterm birth

Introduction

Periodontal disease such as plaque-induced gingivitis and periodontitis has been linked to several systemic conditions such as cardiovascular disease, diabetes, respiratory disease, and adverse pregnancy outcomes (APO), including primarily preterm birth (PTB), low birth weight (LBW), miscarriage, or preeclampsia [1–3]. Although the causative pathophysiological mechanisms behind these relationships remain unclear, a low-grade systemic inflammation with elevated levels of proinflammatory mediators has been proposed to play a key role. Several studies have suggested that patients affected by gingivitis or periodontitis produce higher levels of proinflammatory mediators in gingival crevicular fluid (GCF) compared to healthy subjects, including interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNF-α), and prostaglandin E_2 (PGE_2) [4–6]. Animal studies, which used a subcutaneous tissue chamber model to investigate the effect of a localized challenge of *Porphyromonas*

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gingivalis on the fetoplacental unit, have demonstrated a local increase in bacterial lipopolysaccharides (LPS) and PGE_2, resulting in fetal death and/or fetal growth restriction [7, 8]. Offenbacher et al. postulated that periodontal disease caused by gram-negative anaerobic bacteria may have a negative influence on the course of pregnancy through the production of proinflammatory mediators [9]. Therefore, attempts to use mediators as diagnostic markers to predict pregnancy outcomes have been made [10, 11].

APO, in particular PTB, continue to be a significant public health issue in both developed and developing countries. They represent approximately 10 % of all live births worldwide [12] and account for 28–75 % of all perinatal deaths and over 50 % of all severe developmental disability in children worldwide [13–15]. Therefore, the prevention of APO is an important goal for public health services and thus warranting further research.

Systematic reviews published on the topic of an association between APO and periodontal disease concluded that, currently, despite a consistent association between periodontal disease and PTB/LBW, it is impossible to prove a causative relationship between these two conditions and the findings should be interpreted with great caution until the sources of heterogeneity among studies are more evident [3, 16]. Variability among study designs, diagnostic methods, and definitions of periodontal disease or APO makes it difficult to compare the published data. Although there seems to exist evidence of an association between periodontal disease and APO [3], a recent review was not able to demonstrate reductions in PTB after periodontal therapy in clinical trials [17].

Although, at present, the exact pathophysiological mechanism of a putative causative relationship between periodontal diseases and APO is unknown, the following hypotheses have attempted to provide some explanation [18]:

1. translocation of periodontal pathogens to the fetoplacental unit;
2. influence of LPS from periodontal bacteria on the fetoplacental unit;
3. role of inflammatory mediators (IL-1, IL-6, TNF-α, and PGE_2) in GCF on the fetoplacental unit.

Furthermore, until now, to the best of our knowledge, no systematic review of the literature is available on the potential association between GCF inflammatory mediators and APO. Therefore, the aim of the present systematic review was to analyze the existing literature on the potential association between inflammatory mediators detected in GCF and APO.

Materials and methods

This systematic review has been conducted adhering wherever possible to the guidelines of the PRISMA statement [19] and Needleman [20] by defining a focused question and specific inclusion criteria. An electronic search into two different databases as well as a structured quality assessment was performed. Due to the heterogeneity and variability of the included studies, no meta-analysis could be performed.

Focused question

The focused question "Are elevated GCF inflammatory mediator levels during pregnancy associated with APO?" was defined adhering to the PICO format:

- Population: pregnant women
- Intervention: no intervention was conducted (except for Offenbacher et al. [21])
- Comparison: elevated and non-elevated GCF inflammatory mediator levels
- Outcomes: APO yes/no

Study selection

The inclusion criteria were as follows:

- Studies with pregnant women or women with recent delivery
- Collection of GCF and analysis of GCF inflammatory mediators
- Investigation of APO
- At least 18 subjects included in the study
- Full text written in English
- Study design: randomized controlled trials, case–control studies, or case series

Search strategy

An electronic search into the MEDLINE (PubMed) and EMBASE databases for articles published up to and including April 12, 2012 was performed. The search strategy applied was: "gingival fluid AND pregnancy" (search details: "gingiva" [MeSH Terms] OR "gingiva" [All Fields] OR "gingival" [All Fields]) AND ("body fluids" [MeSH Terms] OR ("body" [All Fields] AND "fluids" [All Fields]) OR "body fluids" [All Fields] OR "fluid" [All Fields]) AND ("pregnancy" [MeSH Terms] OR "pregnancy" [All Fields]). A manual search was performed by screening the reference lists of the selected studies for publications not listed in the electronic databases. A search for gray literature, i.e., possibly relevant but not yet published studies, was also conducted.

Validity assessment

The electronic search as well as the screening of the search results according to the inclusion criteria was independently

conducted by two reviewers (P.S. and R.A.). The manual search and the search for gray literature were performed by P.S. Any possible discrepancies were resolved by discussion between the two reviewers.

Quality assessment

The following points were focused:

- Sample size
- Definitions of APO (were the investigated APO clearly defined?)
- Assessment of APO and description of criteria applied to confirm APO (were adequate means used to confirm APO?)
- Data presentation of GCF mediator levels and GCF sampling methods
- Assessment of periodontal conditions (which diagnostic means were used to assess periodontal disease?)
- Definition of periodontal disease (was a clear definition of periodontal disease described?)
- Management of confounders (was the study performed with a thorough control for confounders?)

Results

Selection process of the included publications

The search into the PubMed and EMBASE databases resulted in the identification of 245 titles, of which 27 titles were identified in both databases. The manual search and the search for gray literature did not result in the detection of further articles or data. Initial screening of these 245 titles resulted in the consideration of 59 abstracts. Independent screening of these 59 abstracts with respect to the inclusion criteria resulted in further consideration of eight publications. For these eight publications, full-text articles were obtained and analyzed. All eight publications finally matched the inclusion criteria (Fig. 1). For each step of the selection process, agreement on the included studies was reached in 100 % between the two reviewers. The studies which remained after the last selection (n=8) are presented in Table 1.

Quality assessment

The sample size varied widely among publications; two studies included 18–30 subjects [22, 23], two studies included 31–60 subjects [24, 25], one study included 61–100 subjects [26], and three studies included 101–128 subjects [21, 27, 28].

Although differences exist among studies concerning the choice of investigated APO, these were always clearly defined; while a majority of the studies reported preterm low birth weight (PLBW) [24, 26–28], one examined PTB and LBW separately [23] and one examined only PTB [21]. Two authors reported preeclampsia [22, 25].

With respect to the description of the criteria applied to confirm APO, three studies were classified as adequate, which presented a clear description of the criteria applied to confirm either PLBW [23] or preeclampsia [22, 25]. Five studies, which did not report how the gestational age was determined in order to confirm PLBW, were classified as unclear [21, 24, 26–28].

Data presentation of GCF mediator levels also differs among publications. A majority of the authors applied adequate methods and reported concentrations [22–26, 28], although it has to be mentioned that only two of these studies presented a time specification concerning the GCF sampling [22, 25]. Two studies were classified as inadequate, reporting either no defined volume and no data about sampling and processing [27] or mass/volume eluent, which results in an irrelevant concentration [21].

Concerning the assessment of periodontal conditions, a majority of the studies applied adequate diagnostic methods based on full-mouth probing measurements [21, 23–25, 27, 28], whereas two studies applied inadequate diagnostic methods conducting only partial mouth recordings such as Community Periodontal Index of Treatment Needs (CPITN) [26] or examining only the Ramfjord index teeth [22].

A clear definition of periodontal disease was presented in five studies [23, 25–28], but not in the other three [21, 22, 24]. Although Offenbacher et al. [24] reported the Extent and Severity Index, this study lacks an explicit definition of thresholds for periodontal disease.

Regarding the management of confounders, only three studies were performed with control for relevant confounders such as maternal age, race, smoking, alcohol intake, drug use, socioeconomic status, genitourinary infections, and previous history of PLBW [21, 25, 28]. Five studies did not report a thorough control for confounders [22–24, 26, 27], although Tarannum et al. [23] excluded patients with several known risk factors for PLBW and, in the study by Oettinger-Barak et al. [22], test and control subjects were matched for age and socioeconomic status (Table 2).

Association between GCF inflammatory mediators and APO

In a study examining 40 women with either PTB/LBW or normal birth weight (NBW), Offenbacher et al. found a consistent association between pregnancy outcome and different periodontal parameters [24]. PLBW mothers showed a slightly worse but not statistically significant periodontal disease status compared with NBW mothers (mean extent scores

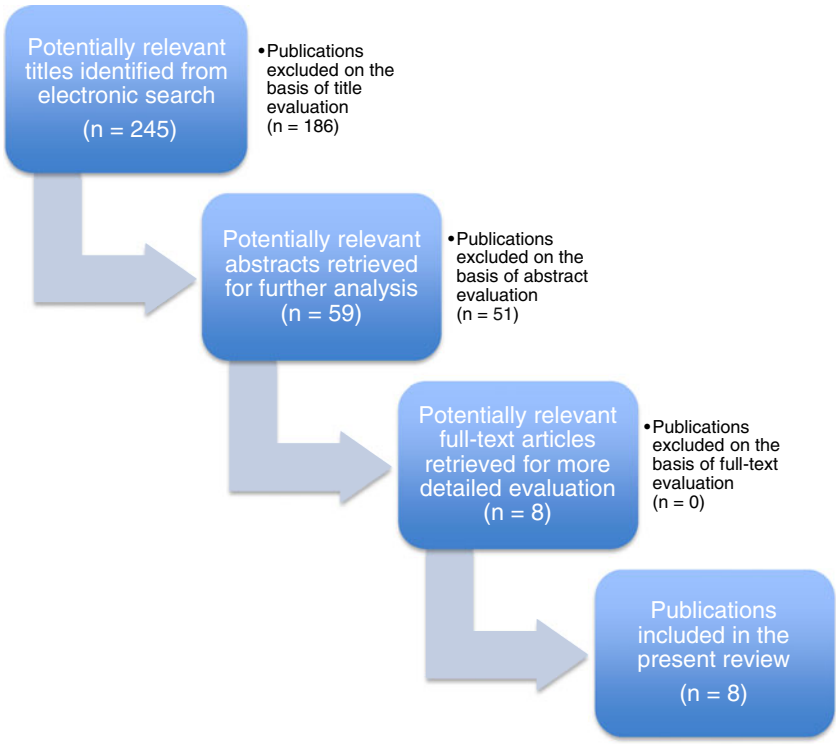

Fig. 1 Search strategy of the relevant publications

(clinical attachment level (CAL) ≥4 mm) of 43 and 40 %, respectively). A significant twofold elevation in the GCF PGE_2 levels and a tendency, although not significant, for elevated IL-1β levels were found in mothers with PLBW as well as significantly higher levels for all tested periodontopathogenic bacteria including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*. In addition, this study reported an inverse relationship between GCF PGE_2 levels and birth weight within primiparous women.

The results of a study with 84 women with PLBW and 44 controls by Konopka et al. showed a significantly higher frequency of periodontitis (chronic and aggressive) in the test group [27]. In all women with PLBW, there were significantly higher levels of IL-1β and PGE_2 in GCF, and in primiparous women, there was also a higher PGE_2 level in the blood serum, compared to the controls. Secretion of these proinflammatory mediators in whole blood after *Escherichia coli* LPS stimulation showed no significant differences between groups. Furthermore, an inverse association between GCF PGE_2 concentration and birth weight in primiparous women was found in this study.

In a case–control study including 92 women, Carta et al. reported significantly worse periodontal disease status as well as significantly higher PGE_2 and IL-1β GCF levels in PLBW mothers compared with NBW controls [26]. In this study, only 10 % of subjects were free of any periodontal disease (CPITN score=0).

Tarannum et al. conducted a pilot investigation including 22 pregnant women with normal pregnancy outcomes and APO and explored the possible use of the GCF PGE_2 level as a risk predictor of PLBW [23]. They reported higher mean GCF PGE_2 levels in mothers of PTB and LBW infants than in mothers of NBW and full-term birth infants, but this difference was not significant.

Noack et al. could not confirm periodontitis as a risk factor for PLBW in a case–control study with 59 pregnant women with a high risk for PLBW and 42 control women [28]. No significant differences in periodontal status and levels of periodontopathogenic bacteria such as *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *P. gingivalis*, *Prevotella intermedia*, and *T. forsythia* between the groups were reported. To be mentioned is a tendency to higher GCF IL-1β levels in the case group, which was not significantly different from controls. The prevalence of moderate to severe periodontitis and generalized periodontitis in this study sample was low.

Concerning a possible relationship between GCF mediators and preeclampsia, several investigations show positive results. Canakci et al. reported a highly significant association between severe periodontitis and mild to severe preeclampsia in a case–control study with 59 pregnant women [25]. A majority of the women in this study presented with mild to severe periodontal disease. All measured mediator levels (IL-1β, TNF-α, and PGE_2) were significantly higher in preeclamptic than normotensive women in both GCF and serum and a significant positive correlation between GCF and serum mediator levels was found. A negative significant correlation was also found between all GCF and serum mediator levels and LBW (except for PGE_2 in serum).

Table 1 Characteristics of included studies

	Study design	Subjects	Time of examination	Type and compartment of mediators examined	Data presentation of GCF mediator levels; GCF sampling method	Periodontal parameters examined (apart from GCF samples)	Definitions of periodontal disease	Periodontal disease status of subjects	APO	Conclusions
Offenbacher et al. 1998 [24]	Case–control study	*N*=40; women immediately before/after delivery: 25 with PLBW or history of PLBW; 15 with NBW and no history of PLBW	Immediately before delivery or within 3 days postpartum	IL-1β, PGE_2 (GCF) →ELISA	Mass/volume GCF;paper strips	PPD, CAL, BOP, subgingival plaque samples	ESI	Mean extent score (CAL ≥4 mm): cases, 42.7; controls, 39.5	PLBW	This study found significantly higher GCF PGE_2 levels in PLBW mothers than in NBW mothers and an inverse association between GCF PGE_2 levels and birth weight
Konopka et al. 2003 [27]	Case–control study	*N*=128; women immediately after delivery: 84 with PLBW; 44 with NBW, FTB	On the third day after labor	IL-1β, PGE_2 (GCF, serum) →EIA	Mass/volume (not defined); no data about sampling and processing	PBI, PPD	Severe, generalized periodontitis: PDI >4 (PDI = PBI + mean PPD + number of PPD >5 mm/number of sextants)	Severe and generalized periodontitis (PDI >4): cases, 39 %; controls, 14 %	PLBW	This study found significantly higher GCF PGE_2 and IL-1β levels in PLBW women compared to controls
Carta et al. 2004 [26]	Case–control study	*N*=92; women immediately after delivery: 46 with PLBW or history of PLBW; 46 with NBW, FTB, and no history of PLBW	Within 48 h from labor	IL-1β, PGE_2 (GCF) →ELISA	Mass/volume GCF;paper strips	PPD	Periodontal health: CPITN=0; severe periodontal disease: CPITN ≥4	Severe periodontal disease (CPITN ≥4): cases, 40 %; controls, 3 %	PLBW	This study concluded that PLBW mothers show significantly higher GCF PGE_2 and IL-1β levels than NBW mothers
Tarannum et al. 2011 [23]	Case series (pilot study)	*N*=22; pregnant women	28–32 weeks of gestation, 1 month after parturition	PGE_2 (GCF, serum) →ELISA	Mass/volume GCF; microcapillaries	OHI-S, CAL, BI	Moderate periodontitis: ≥30 % sites AL ≥3 mm	Clinically healthy periodontium	PTB, LBW	This study found a positive correlation (not significant) between serum and GCF PGE_2 levels and a negative correlation (not significant) between PGE_2 levels and birth outcome
Noack et al. 2005 [28]	Case–control study	*N*=101; pregnant women: 59 with preterm contractions,	Cases: within 12 h after hospitalization; controls:	IL-1β (GCF) →ELISA	Mass/volume GCF;paper strips	PI, PPD, AL, BOP, subgingival plaque samples	Generalized periodontitis (Offenbacher et al. [9]): ≥60 % sites AL ≥3 mm	Mean percentage of sites with AL ≥3 mm: cases, 7.7	PLBW	This study postulates that periodontitis is not a detectable risk factor for PLBW. A tendency to higher GCF IL-1β levels in

Table 1 (continued)

	Study design	Subjects	Time of examination	Type and compartment of mediators examined	Data presentation of GCF mediator levels; GCF sampling method	Periodontal parameters examined (apart from GCF samples)	Definitions of periodontal disease	Periodontal disease status of subjects	APO	Conclusions
		high risk for PLBW; 42 with no preterm contractions, NBW, FTB	within 3 days postpartum					resp. 10.8; controls, 10.6		cases (not significant) was found
Canakci et al. 2007 [25]	Case–control study	*N*=59; pregnant women: 20 with mild preeclampsia; 18 with severe preeclampsia; 21 healthy	Within 48 h preceding delivery	IL-1β, TNF-α, PGE_2 (GCF, serum) →ELISA (IL-1β, TNF-α) →EIA (PGE_2)	Mass/volume GCF;paper strips (30 s)	PI, PPD, CAL, BOP	Periodontal health: no sites PPD ≥4 mm; mild periodontal disease: 1–15 sites PPD ≥4 mm + BOP; severe periodontal disease: ≥15 sites PPD ≥4 mm + BOP	Severe periodontal disease: cases, 72.2 resp. 50.0 %; controls, 33.3 %	Mild/severe preeclamp-sia	This study postulates that periodontitis is associated with an increased risk for preeclampsia. IL-1β, TNF-α, and PGE_2 levels were significantly higher in preeclamptic than normotensive women
Oettinger-Barak et al. 2005 [22]	Case–control study	*N*=30; pregnant women: 15 with preeclampsia; 15 healthy	Up to 48 h prior to delivery	IL-1β, TNF-α, PGE_2 (GCF) →ELISA	Mass/sample; paper strips (30 s)	PI, GI, GO, PPD, GR	Not reported	Mean PPD/ CAL: cases, 2.98/ 3.33 mm; controls, 2.11/ 2.30 mm	Preeclampsia	This study found significantly greater periodontal breakdown and significantly higher GCF IL-1β, TNF-α, and PGE_2 levels in the preeclampsia group compared to controls
Offenbacher et al. 2006 [21]	Randomized, delayed-treatment, controlled pilot trial	*N*=109; pregnant women: 56 with intervention; 53 with delayed-treatment	Intervention group: second trimester; delayed-treatment group: 6 weeks post partum	IL-1β, PGE_2, 8-iso, IL-6 (GCF) IL-6, sICAM1, 8-iso, sGP130, IL-6 sr, CRP (serum) →ELISA	Mass/volume eluent (200 μl); paper strips	PI, GI, PPD, BOP, GR, subgingival plaque samples	Not reported	Mean PPD/ AL: intervention group, 2.27/ 0.55 mm; controls, 1.98/ 0.54 mm	PTB	This study postulates that periodontal treatment during pregnancy leads to a significant reduction in the rate of PTB and a significant decrease in GCF IL-1β levels

AL attachment loss, *BI* bleeding index, *BOP* bleeding on probing, *CAL* clinical attachment level, *CPITN* Community Periodontal Index of Treatment Needs, *CRP* C-reactive protein, *EIA* enzyme immunoassay, *ELISA* enzyme-linked immunosorbent assay, *ESI* Extent and Severity Index, *FTB* full-term birth, *GCF* gingival crevicular fluid, *GI* gingival index, *GO* gingival overgrowth, *GR* gingival recession, *IL-1β* interleukin-1β, *IL-6* interleukin-6, *IL-6sr* interleukin-6 soluble receptor, *8-iso* 8-isoprostane, *LBW* low birth weight, *NBW* normal birth weight, *OHI* oral hygiene instruction, *OHI-S* Oral Hygiene Index—Simplified, *PBI* Periodontal Bleeding Index, *PDI* Periodontal Disease Index, PGE_2 prostaglandin E_2, *PI* plaque index, *PLBW* preterm low birth weight, *PPD* probing pocket depth, *PTB* preterm birth, *sGP130* soluble glycoprotein 130, *sICAM1* soluble intercellular adhesion molecule 1, *SRP* scaling and root planing, *TNF-α* tumor necrosis factor-alpha

Table 2 Quality assessment of included studies

	Sample size	Clear definition of APO	Description of criteria applied to confirm APO	Data presentation of GCF mediator levels; GCF sampling method	Method for assessment of periodontal conditions	Clear definition of periodontal disease	Controlling for confounders
Offenbacher et al. 1998 [24]	*N*=40	Yes (PLBW)	No	Mass/volume GCF; paper strips	Full-mouth assessment	No (ESI)	No
Konopka et al. 2003 [27]	*N*=128	Yes (PLBW)	No	Mass/volume (not defined); no data about sampling and processing	Full-mouth assessment	Yes (severe, generalized periodontitis: PDI >4 (PDI = PBI + mean PPD + number of PPD >5 mm/number of sextants))	No
Carta et al. 2004 [26]	*N*=92	Yes (PLBW)	No	Mass/volume GCF; paper strips	CPITN	Yes (periodontal health: CPITN=0; severe periodontal disease: CPITN ≥4)	No
Tarannum et al. 2011 [23]	*N*=22	Yes (PTB, LBW)	Yes	Mass/volume GCF; microcapillaries	Full-mouth assessment	Yes (moderate periodontitis: ≥30 % sites AL ≥3 mm)	No
Noack et al. 2005 [28]	*N*=101	Yes (PLBW)	No	Mass/volume GCF; paper strips	Full-mouth assessment	Yes (generalized periodontitis (Offenbacher et al. [9]): ≥60 % sites AL ≥3 mm)	Yes
Canakci et al. 2007 [25]	*N*=59	Yes (mild/severe preeclampsia)	Yes	Mass/volume GCF; paper strips (30 s)	Full-mouth assessment	Yes (periodontal health: no sites PPD ≥4 mm; mild periodontal disease: 1–15 sites PPD ≥4 mm + BOP; severe periodontal disease: ≥15 sites PPD ≥4 mm + BOP)	Yes
Oettinger-Barak et al. 2005 [22]	*N*=30	Yes (preeclampsia)	Yes	Mass/sample; paper strips (30 s)	Ramfjord teeth	No	No
Offenbacher et al. 2006 [21]	*N*=109	Yes (PTB)	No	Mass/volume eluent (200 μl); paper strips	Full-mouth assessment	No	Yes

AL attachment loss, *BOP* bleeding on probing, *CPITN* Community Periodontal Index of Treatment Needs, *ESI* Extent and Severity Index, *LBW* low birth weight, *PBI* Periodontal Bleeding Index, *PDI* Periodontal Disease Index, *PLBW* preterm low birth weight, *PPD* probing pocket depth, *PTB* preterm birth

Oettinger-Barak et al. examined 15 healthy pregnant women and 15 with preeclampsia and found significantly greater periodontal breakdown as well as significantly higher GCF levels of proinflammatory mediators IL-1β, TNF-α, and PGE_2 in the preeclampsia group compared to controls [22]. Furthermore, periodontal parameters (probing pocket depth (PPD) and CAL) correlated partially with mediator levels in GCF.

Investigating the effect of periodontal therapy on GCF mediator levels and pregnancy outcomes, Offenbacher et al. conducted a randomized, delayed-treatment, controlled pilot trial [21]. They evaluated the effects of scaling and root planing during the second trimester and the use of a sonic power toothbrush on the rate of PTB and clinical parameters such as oral pathogens, GCF, and serum levels of proinflammatory mediators and changes in periodontal status. Their results showed that periodontal therapy lead to significant improvements in clinical parameters and significantly decreased GCF IL-1β levels and the odds ratio for PTB.

Discussion

The present systematic review has attempted to evaluate the existing literature concerning a potential association between GCF inflammatory mediators and APO. The findings of the present systematic review suggest a positive association between GCF inflammatory mediators and APO. The majority of the included studies confirmed a significant positive association between inflammatory mediators such as IL-1β, PGE_2, and TNF-α in GCF and either PLBW [23, 24, 26, 27] or preeclampsia [22, 25]. Two studies were not able to prove a significant relationship between GCF mediator levels and PLBW in spite of a tendency for higher GCF IL-1β and PGE_2 levels in PLBW women compared to controls [23, 28]. The degree of methodological quality of the studies was not crucial for the outcome being significant or nonsignificant. The low prevalence of moderate to severe periodontitis and generalized periodontitis in the study by Noack et al. [28] and the inclusion of only women with clinically healthy gingiva in the study by Tarannum et al. [23] might have influenced these results. In addition, several studies have shown an inverse association between GCF PGE_2, IL-1β, or TNF-α levels and birth weight [24, 25, 27]. These results appear to indicate that periodontal disease during pregnancy may result in enhanced systemic exposure of inflammatory mediators of oral origin, which may contribute to APO.

The heterogeneity of outcomes among selected studies may be due to several possible sources. First of all, different APO were defined and investigated (PLBW, PTB, LBW, and preeclampsia). Analyzing GCF, different inflammatory mediators were reported (mainly PGE_2, IL-1β, and TNF-α). In addition, different methods of data presentation of GCF mediator levels were used. While a majority of the authors reported concentrations (mass/volume GCF or mass/sample) of mediator levels, some did not measure GCF volume and, therefore, were not able to report relevant concentrations. Methods for the assessment of periodontal conditions and definitions of periodontal disease varied widely and only few studies reported distinct thresholds for the definition of periodontal disease. Furthermore, the periodontal disease status of the included subjects showed high diversity. Only three studies conducted a thorough control for potential confounders. Adjustment for confounding variables is of high importance to avoid study bias. Thus, the association between periodontitis and PLBW supported in some studies may be due to inadequate adjustment. Due to the heterogeneity and variability of the available studies, no analysis could be performed evaluating the association between GCF mediators and APO.

The previously discussed findings appear to support the hypothesis that an association of periodontal disease and APO could result from the action of a periodontal reservoir of inflammatory mediators (IL-1, IL-6, TNF-α, and PGE_2) on the fetoplacental unit. This hypothesis is further supported by studies which suggested that PGE_2 and cytokines such as IL-1 and TNF-α play an important role in the normal physiologic process of parturition as well as in pathologic prematurity [29–32]. IL-1β is a potent stimulator of the prostaglandin synthesis by decidua and amnion and was the first cytokine implicated in the onset of labor in the presence of infection [33–35]. Amniotic fluid levels of PGE_2 rise steadily throughout pregnancy until a critical threshold level is reached to induce labor, cervical dilation, and delivery [36]. Romero et al. observed elevated levels of PGE_2 as a consistent and reproducible fact of PTB, even in the absence of clinical or subclinical genitourinary tract infection and they postulated that the majority of PTB cases are probably caused by an infection of unknown origin [37]. In addition, elevated proinflammatory cytokines in amniotic fluid such as TNF-α or IL-6 are related to severe neurological outcome in preterm neonates, including periventricular leukomalacia and cerebral palsy.

However, a linear causal relationship between GCF inflammatory mediators or periodontal disease in general and APO has not yet been demonstrated. Controversial hypotheses do not support a linear causal relationship but rather suggest some common features between periodontal disease and APO, mainly based on genetically determined individual predisposition to yield a hyperinflammatory response to bacterial challenge [38, 39]. This hyperinflammatory response may in turn alter the patient's inflammatory phenotype, thus placing the patient at risk for both disorders. This possibility gives rise to some new questions: Does advanced periodontitis rather play a role as a susceptibility marker for

APO than as a causal factor? Does a genetically determined hyperinflammatory response that predisposes an individual to periodontal disease also predispose to other multifactorial diseases which are related to inflammation?

The hypothesis of a genetically determined hyperinflam matory trait appears to be supported by the results of a study by Damaré et al. who stated that, despite a significant positive association between PGE_2 levels in GCF and amniotic fluid, the low levels of PGE_2 in amniotic fluid do not support the assumption of a translocation of GCF PGE_2 to the fetal placental unit [40]. Thus, it seems that the patient-specific state of systemic responsiveness and monocytic response determines both the GCF and amniotic fluid PGE_2 levels. They concluded that GCF levels of PGE_2 may be used to provide an indirect estimate of the amniotic fluid levels of PGE_2 and may serve as a risk factor, since amniotic fluid PGE_2 levels can be predictive of preterm delivery.

Converse conclusions come from the study by Konopka et al. [27] who could not confirm a higher secretion of IL-1β and PGE_2 in whole blood after bacterial LPS stimulation in PLBW women. This lack of significant differences suggests that more frequent occurrence of a hyperactive cell phenotype synthesizing these mediators is rather not responsible for PLBW. In contrast, they found a significantly higher secretion of IL-1β after stimulation in patients with gingivitis and periodontitis compared to subjects with a healthy periodontium, apparently indicating that IL-1β secretion is mainly due to exposition of special cells to bacterial products and that PLBW cannot be related with frequent incidence of a hyperactive cell phenotype.

The conflicting results on the causal relationship between GCF inflammatory mediators and APO may be explained by the fact that the majority of the studies investigating a correlation between mediator levels in GCF and serum either failed to show a statistically significant correlation between mediator levels in GCF and serum or they only found a weak statistically not significant correlation [23, 41]. Only Canakci et al. reported a statistically significant correlation between GCF and serum mediator levels [25]. Fiorini et al. [41] found a low correlation between GCF and serum mediator levels. Consequently, their results have failed to support the hypothesis that periodontal disease may lead to a low-grade systemic inflammatory status in pregnant women. On the other hand, a limitation of the mentioned study is the fact that severe periodontal destruction was only detected in a small percentage of the subjects and, thus, it cannot be excluded that the results might have been influenced by including only women with severe periodontitis.

The findings of the study by Fiorini et al. are in agreement with other studies which also found low serum levels of IL-1β, IL-6, IL-8, and TNF-α in pregnant women [21]. Few other studies have assessed concomitantly GCF and systemic inflammatory mediator levels independent of pregnancy. Orozco et al., who evaluated serum and GCF mediator levels in 10 gingivitis and 10 periodontitis patients, found detectable levels of IL-1β only in GCF [42]. Trombelli et al. also reported similar results of IL-1β in experimentally induced gingivitis [43]. Fentoglu et al. evaluated the serum and GCF levels of IL-1β, IL-6, and TNF-α in 123 hyperlipidemic patients and 68 systemically healthy controls and found consistently higher serum than GCF levels of all mediators evaluated, irrespective of periodontal status or lipid status [44].

Offenbacher et al. concluded that periodontal therapy during pregnancy leads to clinical improvements such as reduction of plaque index, gingival index, and PPD and lower GCF levels of PGE_2 and IL-1β [21, 45]. It could be confirmed that initial periodontal treatment during pregnancy leads to a significantly decreased incidence odds ratio for PTB and that periodontal treatment during pregnancy is safe. The reported finding that periodontal treatment is associated with decreased GCF mediator levels is in agreement with a recent study, which examined a wide range of GCF mediators including IL-1α, IL-1β, IL-6, and IL-8 [46]. The fact that periodontal treatment has been shown to lead to decreased GCF mediator levels and PTB incidence further supports the hypothesis of a linear causal relationship between GCF mediators and APO. On the other hand, it should be kept in mind that recent studies have failed to confirm a statistically significant decrease in the incidence of PTB, despite the fact that they did not investigate proinflammatory mediators in GCF [47, 48] and are also supported by the conclusion of a recent systematic review, indicating that treatment of maternal periodontal disease does not seem to decrease the risk of PTB and/or LBW [17].

Conclusions

Taken together, the present findings indicate that:

(a) A positive association between GCF inflammatory mediator levels and APO/PTB might be present, but the results need to be considered with great caution because of the heterogeneity and variability among the studies.

and

(b) Further studies with an adequate number of patients, allowing for an appropriate analysis, are warranted to definitely confirm this association.

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