Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2020 Oct 23; 8(F):253-259.
https://doi.org/10.3889/oamjms.2020.5409
eISSN: 1857-9655
Category: *F - Review Articles*
Section: *Narrative Review Article*

Exploring the Role of *Fusobacterium nucleatum* in Preterm Birth: A Narrative Review

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Edited by: Eli Djulejic
Citation: Rapone B, Ferrara E, Converti I, Loverro M, Loverro MT, Gnoni A, Petruzzi M, Corsalini M, Scacco S, Di Naro E. Exploring the Role of *Fusobacterium nucleatum* in Preterm Birth: A Narrative Review. Open Access Maced J Med Sci. 2020 Oct 23; 8(F):253-259. https://doi.org/10.3889/oamjms.2020. 5409
Keywords: Periodontal disease; Oral microbiome; Pregnancy; Pregnancy outcomes; Preterm birth; *Fusobacterium Nucleatum*
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Received: 03-Sep-2020
Revised: 09-Oct-2020
Accepted: 13-Oct-2020
Funding: This research did not receive any financial support
Competing Interest: The authors have declared that no competing interest exists

Abstract

In recent years, substantive attention has been drawn to the relationship between oral microbiome homeostatic equilibrium disruption and systemic health, demonstrating the negative impacts of this reciprocal biological interplay. Increasingly, there is a concern over the potential noxious effect of oral microbiome dysbiosis on obstetric poor outcomes, focusing on preterm birth. This epidemiological observation remains unexplained, although biologically plausible mechanism has been proposed. Intrauterine infection has long been associated with adverse pregnancy, when the elicitation of an immune response is determinant. There is evidence that *Fusobacterium nucleatum* (FN), a Gram-negative anaerobe ubiquitous in the oral cavity, infects the mouse placenta originating in the decidua basalis. Based on the current data in literature, we performed a review to provide resources for the explanation of the potential impact of microbiome dysbiosis on poor obstetric outcomes, focusing on the role of FN.

Introduction

Periodontal disease and oral microbiome

It has been increasingly recognized that alteration of oral microbiome is responsible for modulating systemic disorders at least might be partially caused by periodontal pathogens translocation [1], [2], [3]. The establishment of microbiome dysbiosis can exert detrimental effects on the immune system. Several systemic disorders have been intimately blended with periodontal inflammation, but its relation to poor obstetric outcomes is less convincing [4], [5], [6], [7]. It is ascertained that the periodontitis is related to the elevation of systemic levels of a specific inflammatory marker (C-reactive-protein) and pro-inflammatory cytokines [8]. This association is based on the range of the evidence furnished by the empirical studies which have supported this assumption [9], [10], [11]. For example, in the seat of diabetes, it has been observed a deleterious impact of periodontitis on glycemic control for by enhancing the general immune responsiveness [12]. It has been established that the oral microbiota is critically modified during diabetes, and the disruption of oral microbiome eubiosis [13], [14], [15] determines a feedback loop in which host and cell components and metabolites of microbial community regulate each other, leading to a perpetuation of the dysbiotic condition [16], [17], [18]. It seems probable that analogous results would be produced on the amniotic fluid of pregnant women [19]. Any interference which its internal action may show an immune reactivity [20], [21], [22]. The oral microbiome during periodontitis is characterized by lower phylogenetic diversity and pathobiont overgrowth [23]. The exciting cause of this affection is a dysregulation of the oral microbiome. This enables reason to conceptualize the role of periodontal infection process in abstraction from the restriction of limited confined infection as the condition of its ability of sustaining a low-grade systemic inflammatory status [24]. The construction of this theory and expansion of the role of peripheral inflammation has posed essential questions for analysis of the dysregulation of oral microbiome [25], [26]. Therefore, the potential damaging effect of periodontal pathogens in pregnant women is its tendency to cause low-grade systemic inflammation,

which may lead to premature rupture of membranes [18]. Whether the impact from periodontal infection influences or explicates a direct effect is uncertain but suggests a potentially damaging interaction between periodontitis and preterm delivery (PTD) [23]. From the empirical findings, a clear interest focused on the placental inflammation has emerged [1], [4], [25], [26], [27], [28], [29]. Central relationship between the periodontal disease and preterm birth (PTB) is due to an inflammatory response system responding to signal from bacterial invasion [30], [31]. Interestingly, a similarity between placental and oral microbiome was found to be essential to describe as coherent a hypothesis of relationship between oral dysbiosis and pregnancy poor outcomes [32], [33], [34], [35], [36], [37]. This hypothesis is referred to an activate inflammatory signaling pathways stimulated by periodontal damage, or direct dissemination of periodontal pathogens through hematogenous spread from the oral cavity [18], [21], [27], [35], but they do not fully explain such behaviors, suggesting that other mechanisms and factors might play a key role in determining the link [22], [23], [24], [25]. *Fusobacterium nucleatum* (FN) is an opportunistic commensal Gram-negative anaerobe microorganism ubiquitous to the oral cavity, one of the most abundant pathogens implicated in severe periodontal disease [18], [38], [39], [40], [41], [42], [43], [44], [45], [46]. A collective body of evidence suggests an interconnection in times of homeostasis and disease and reveals a highly prevalence of this oral pathogen in intrauterine infection. FN has been isolated from the amniotic cavity, placenta, chorioamniotic membranes of women delivering prematurely, and fetus. Further, it has been identified in the blood vessels in murine placentas [47]. However, *Fusobacterium* spp. is common isolate in vaginal microbiota, representing a potential source of amniotic fluid colonization.

The relationship between the oral microbiome and PTB

The pathogenesis of PTB is composite and only incompletely identified [1]. In addition to a genetic susceptibility, the main elements responsible for the premature delivery include environmental factors and infectious agents [2], [3], [36], [37], [38]. Increased expression of the Th1 cytokines IL-2 and IL-12, and IFN gamma in placentas from the PTD and premature rupture of membranes women has been observed [39], [40], [41], [42], [43], [44], [45], and there is increasing evidence that the pathogenic colonization of the intrauterine space leads to placental high-grade inflammatory response [40]. In recent years, substantive attention has been drawn to the relationship between the oral microbiome homeostatic equilibrium disruption and systemic health, demonstrating the negative impacts of this reciprocal biological interplay [41], [43]. In this novel specific context, several researchers have an interest in analyzing the meaning of the potential impact of oral microbiome variations on poor pregnancy outcomes [45], [46], [47]. However, the actual models concerning the biological mechanism underlying the potential relationship between oral microbial community and PTB concern the oral microbiote role, whereby the oral microbiote might be responsible for the colonization of placental membranes and basal plate decidua [11], [12], [13], [14], [15]. A multitude of studies has identified potential periodontal pathogens that might have a causal role in determining the risk of PTB, evaluating the combined influences of genes with intrinsic and extrinsic environmental factor [46], [47], [48], [49]. The balance of the oral microbiome is conditioned on the biological resources, environmental modifications, and the development of *sui generis* species variety. Patients with persistent periodontitis have a chronic immune reaction which reflects increments in the systemic concentration of tumor necrosis factor-α, IL-1β, IL-6, and C-reactive protein among other inflammatory mediators [16], [33], [49]. Thus, anomalous immunoregulatory circuit can contribute to the etiology of inflammation-related disease [23], [33], [49], [50], [51]. The neuroendocrine/immune system interaction during pregnancy is part of broadly physiological events with the panoply of associated alterations, involving also the oral microbiote composition [16], [17], [32], [52]. The exposure to these hormonal changes is postulated to trigger the oral microbial community shift, resulting in the manifestation of more disruptive periodontitis [18], [19], [20], [21], [53], [54], [55], [56]. This transition may provide an explanation of understanding the relationship between periodontal pathogens and PTB [56], [57], [58], [59].

The linkage between FN and PTB

The oral cavity encompasses a number of bacteria that share many virulence characteristics and that may be capable of causing a low-grade systemic inflammation [16], [17], [18], [19], [31], [45], [48], [59]. However, instead of contributing on systemic alterations, some periodontal pathogens seem to disseminate overcoming the placental barrier leading to localized placental inflammation, triggering preterm membrane rupture [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [45], [51]. The oral microbiome in pregnancy has been widely investigated to study the contributions of potential translocated periodontal pathogens to PTB risk [18], [29]. Specifically, it has been investigated the role of the Gram-negative periodontal pathogen FN, an opportunistic pathogen agent having a specific role in the pathogenesis of different periodontal conditions, and it is increasingly recognized as an important agent of PTB [42], [50]. FN is credited with the distinction of being the most crucial pathogen implicated in the pathogenesis of periodontitis [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36], [37], [38], [39], [40], [41], [42], [43], counting its systemic effects on immune system

[59], [60]. The localized infection and inflammation of the mouse placenta mediated by oral FN colonization have been described previously by several researchers who suggested that the hematogenous transmission of this bacterium had a detrimental effect on pregnancy, producing intrauterine inflammatory response [51]. FN has been isolated from amniotic fluid at a different gestational age [52], [53]. Concurrent chorioamnionitis at term with intact membranes has been associated with the presence of FN in the amniotic fluid. However, these reports, although no descriptions of underlying mechanism were given, confirm the presence of the FN suggesting a need of clarification of its role [52], [53]. The human oral microbiome represents a dynamic ecosystem comprising >700 different species [52], [61]. The colonization characteristics of pathogenic bacteria in the placenta are important to understand their potential role in PTB risk [54], [62]. FN is the type species of the genus *Fusobacterium*, belonging to the family *Bacteroidaceae* [12], [43]. FN is categorized into five subspecies (FN subsp. nucleatum, FN subsp. polymorphum, FN subsp. fusiforme, FN subsp. vincentii, and FN subsp. animalis) on the basis of the several phenotypic characteristics and DNA homology. It is a Gram-negative, non-spore-forming obligate anaerobe, having a G1C content of 27–28 mol% and a genome size of about 2.4 3 106 bp. It is one of the most common periodontal species associated with periodontitis and it has already been adverted for its power to spread over a periodontal site and establish an efficient systemic inflammatory response [27], [28]. This linkage may be determined by the ability of the pathogen to colonize the placenta, through its ability to adhere and invade the host epithelial and endothelial cells through FadA adhesin, a virulence factor, which may be involved in pathogenesis of intrauterine infection [26], [27], [28], [29], [30]. It has been suggested that the subsequent affection of the placenta may be probably induced through colonization, exciting the premature rupture of fetal membranes, because of the inflammation may assume an unhealthy character, in consequence of the severity of the infection [33], [34], [35]. The most conventional model describing the relevant biological mechanism underlying the relationship between oral pathogens and PTB is highly empirical. The hypothesis, therefore, often describes based on hematogenous spread to explain the involvement of oral microbiota as cause of intrauterine infection [32], [36]. Compelling evidence supporting systemic exposure to the Gram negative derived the lypopolysaccharides, responsible for much of the toxicity of pathogen bacteria, characterized by the ability to trigger a cascade of responses ultimately. The role of FN virulence factors in placenta invasion was verified by deletion and complementation experiments [12]. The FN invasion triggers the Toll-like receptor 4-mediated activation of localized inflammatory response, responsible of preterm membrane rupture [53]. In the closed emerging link, the potential interdependence existing between FN and PTB has been amply explored. Besides the relevance of FN, extensively examined in several available articles, it has been discussed a relevant issue that is strictly related to FN genomics, and genetic significance of heterogeneity, particularly based on metagenomic approach, to evaluate FN's contribution to PTB, and the genetic magnitude of FN heterogeneity, which is intrinsically linked to virulence aspects.

Pathogenic Mechanism of FN: The role of Fusobacterium adhesin A (FadA)

The association with PTB has been widely investigated, emphasizing the ability of Fn to disseminate into the organism adhering and invading endothelial, through the binding to the vascular endothelial (VE) cadherin (CDH5) [16], [33]. The ability of the pathogen to colonize the placenta may be determined by its facility to adhere and invade host epithelial and endothelial cells through a FadA adhesin, a virulence factor, which may be involved in pathogenesis of intrauterine infection [18], [19]. The adhesin FadA is a virulence factor isolated from FN, occurring in two forms: The intact non-secreted form pre-FadA, composed of 129 amino acid (aa) residues and mature FadA monomer (mFadA) secreted form mFadA of 111 aa. The combination of these monomers leads to the constitution of the active complex FadAc, characterized by heterogeneous filaments. Cristal structure of FadA adhesin reveals an intercellular oligomerization mechanism for bacterial aggregation [28], [29], [30]. Studies aimed to elucidating the process of FN translocation into host cells, include genetic analysis defining components that are required for FadA activity [18], [21], [30]. This is exemplified by the identification of the VE-cadherin as a potential FadA receptor. FadA has been identified to play critical role for strong bacterial surface proteins binding and invasion of host cells [16], [19]. This binding is conferred by the minor fimbrial component. It was found that the binding of FadA (for *Fusobacterium* adhesin A) to epithelial cadherin (E-cadherin) is required for bacterial adhesion and invasion of host cells [35], [38]. It has been demonstrated that FadA adhesion gene (fadA) may be a microbial determinant contributing to placental invasion, causing a localized inflammatory response, responsible for fetal demise prematurely [29], [30], [31], [32]. The role of *Fusobacterium* adhesion A in PTB was first reported in pregnant mice infected with FN [37], [38], [39], [40], [41], [54], [59], [60], [61]. It was the first genetic complementation study which confirmed that the promotion of cellular invasion and placental colonization mediated by the exposure of FadA, through construction of FN 12230 USF81, a fadA-complementing clone. In this study, the mechanisms by which EAEC 042 triggers this inflammatory event were assessed. The immunofluorescent staining analysis of expression of FadA on the FN 12230 USF81 strain, fad-A-complementing clone, confirmed that FadA is equally exposed on the bacterial cell surface, promoting

adherence and invasion of host cells [59], [60], [61]. This study provided the first evidence that the expression of FadA was correlated to placental colonization *in vivo* [60]. Studies aimed at elucidating the process of FN translocation into host cells, include genetic analysis defining components that are required for FadA activity [32]. This is exemplified by the identification of the VE-cadherin as a potential FadA receptor [33].

Conclusions

As stressed above, the capacity of translocation of periodontal pathogens is the prerequisite for establishing the relationship, because instead of contributing on systemic alterations, some periodontal pathogens seem to disseminate overcoming the placental barrier leading to localized placental inflammation, triggering preterm membrane rupture [25], [26]. Specifically, it has been investigated the role of the Gram-negative periodontal pathogen FN, an opportunistic pathogen agent having a specific role in pathogenesis of different periodontal conditions [1], [4], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26], and it is increasingly recognized as an important agent of PTB. A multitude of studies has identified potential periodontal pathogens that might have a causal role in determining the risk of PTB, evaluating the combined influences of genes with intrinsic and extrinsic environmental factors [45], [46], [47], [48]. These investigations have been pivotal in providing resources for the explanation of the potential impact of microbiome dysbiosis on poor obstetric outcomes [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26]. It has been increasingly recognized that alteration of oral microbiome is responsible for modulating systemic disorders [25], [26]. Meta-analytic evidence supports the concept of a biunivocal relationship between periodontal infection and systemic diseases as measured by inflammatory markers level across a wide range of biomarkers [35], [36], [37], [38], [39], [40], [41], [42], [43], [44], [45], [46]. Longitudinal studies reveal the aforementioned correlation with a broad spectrum of systemic diseases, such as cardiovascular diseases, neurodegenerative disorders can worsen because of establishing low-grade inflammatory process driven by dysbiosis of oral microbiome. Although ample studies have [15], [21], [22], [23], [24], [25], [26], [27] demonstrated the effect of periodontal infection on systemic immune response, there is still a paucity of information regarding the interaction between periodontitis and poor obstetric outcomes [51], [52], [53], [54], [55], [56]. However, substantially less is known about the potential association of periodontitis with PTB [18]. That said, the most recent research into the potential correlation of noxious effects of oral microbiome dysbiosis in the host microbiome crosstalk and poor obstetric outcomes [39] has concluded that this interconnection has substantial support, but the general ecosystem destabilization during pregnancy and potentially other factors has be yet to be elucidate [43]. The study of PTB-periodontitis relation has long been interested in the way in which two actors can enhance mutual gains [22], [27], [38]. Despite the extensive research intended to achieve this objective, there is still a great deal of ambiguity as to the periodontal pathogen sustain this outcome. Whereas the investigation of periodontal pathogen [21] is largely restricted to the research arena, the use of short-read sequencing approach is not well established in clinical periodontology [45], [46], [47], [48], [49], [50], [51], [52], [53], [54], [55], [56]. Through analyzing the dynamic character of the oral microbial communities and their interactions with the organism, we sought to reveal through which way the association between periodontal disease and general health is understand and interpreted, focus on the relationship between oral microbiome and pregnancy [11]. There has been considerable debate as to whether the oral microbiome should be seen [13] as a risk factor for PTB and whether this induced [26] development of new care strategies in response to the changed scientific discoveries [60]. However, it seems clear that the new conceptualization has critical function through the idea of overlapping microbial community specificity and pro-inflammatory response [13], [15]. Further evidence for the assumption that the oral microbiome alteration may trigger a specific systemic reaction can be adduced by considering the period before the emergence of modern concept of this relationship [1], [2], [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13], [14], [21]. The notion concerning the role of transient microbiome is a relatively recent phenomenon, because the majority of researchers pointed out on the role of pro-inflammatory cytokines only [52]. An integrated model that explains the connection remains absent. The transdisciplinary approach that considers the dynamism and complexity of oral microbial communities [49] might be the key to describing pathways to explain the impact of periodontitis on PTB. Thus, understanding of this influence is pivotal in the determination [32] of whether the criterion of this heuristic concept is applicable, which can be of assistance in determining a subversion of traditional knowledge on systemic pathologic conditions [50].

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