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Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans in umbilical cord and peripheral blood, saliva, colostrum from women with gingivitis

***Porphyromonas gingivalis e Aggregatibacter actinomycetemcomitans* em sangue do cordão umbilical e periférico, saliva e colostro de mulheres com gengivite**

***Porphyromonas gingivalis* y *Aggregatibacter actinomycetemcomitans* en sangre de cordón umbilical y periférico, saliva y calostro de mujeres con gingivitis**

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Abstract

This study investigated and compared the presence of *Porphyromonas gingivalis* (Pg) and *Aggregatibacter actinomycetemcomitans* (Aa) in biological samples of women submitted to the term delivery diagnosed with gingivitis and healthy periodontium. Oral clinical exams were performed in 48 puerperal women including 28 women with healthy periodontium, 15 with gingivitis and 5 with periodontitis (that were excluded of analysis). Samples of umbilical cord blood (CB), peripheral blood (PB), saliva (SA) and colostrum (C) were collected from eligible women after delivery. DNA extraction was accomplished, and the presence of bacteria was detected by PCR with primers and specific probes for each microorganism. Data about previous and current pregnancies and known risk factors were obtained from patients' medical records. Social, demographic, and oral care data was analyzed by interview. All salivas of participants with gingivitis presented Pg and Aa, while a minority of samples of with healthy periodontium had those bacteria ($P<0.05$ for each). Sixty percent of the women with gingivitis presented Pg in the blood samples (PB and CB, $p<0.05$) and 46.6% presented in C. Aa did not present in the blood samples, but 46.6% of women with gingivitis had Aa in the C. No association between socioeconomic and oral health data, detection of microorganisms and presence of the disease. Gingivitis was related to the positive detection of both microorganisms in saliva. Women with gingivitis presented Pg in PB and CB. The presence of Pg and Aa in colostrum occurred only in pregnant women with gingivitis.

Keywords: *Porphyromonas gingivalis; Aggregatibacter actinomycetemcomitans*; Umbilical cord blood; Saliva; Colostrum.

Resumo

Este estudo investigou e comparou a presença de *Porphyromonas gingivalis* (Pg) e *Aggregatibacter actinomycetemcomitans* (Aa) em amostras biológicas de mulheres que tiveram parto a termo com diagnóstico de gengivite e sem doença periodontal. Exames clínicos orais foram realizados em 48 puérperas, incluindo 28 mulheres com periodonto saudável, 15 com gengivite e 5 com periodontite (que foram excluídas da análise). Sangue do cordão umbilical (CU), sangue periférico (SP), saliva (SA) e colostro (C) foram coletados de mulheres elegíveis após o parto. Foi realizada extração de DNA e em seguida a presença de bactérias foi detectada por PCR com primers específicos. Dados sobre gestações anteriores e atuais e fatores de risco conhecidos foram obtidos dos prontuários médicos dos pacientes. Os dados sociais, demográficos e de higiene bucal foram analisados por entrevista. Os resultados mostraram que todas as SA das participantes com gengivite apresentou Pg e Aa, enquanto a minoria das amostras com periodonto saudável apresentou tais bactérias (P <0,05 para cada uma). 60% das mulheres com gengivite apresentaram Pg nas amostras de sangue (CU e SP, p <0,05) e 46,6% apresentaram no C. Aa não apareceu nas amostras de sangue, mas 46,6% das mulheres com gengivite apresentaram Aa em C. Não houve associação entre dados socioeconômicos e de saúde bucal, detecção de microrganismos e presença da doença. Em conclusão, a gengivite esteve relacionada com a detecção positiva de ambas as bactérias em SA. Mulheres com gengivite apresentaram frequentemente Pg nas amostras sanguíneas. A presença de Pg e Aa em C ocorreu apenas em gestantes com gengivite.
Palavras-chave: *Porphyromonas gingivalis; Aggregatibacter actinomycetemcomitans*; Sangue do cordão umbilical; Saliva; Colostro.

Resumen

Este estudio investigó y comparó la presencia de *Porphyromonas gingivalis* (Pg) y *Aggregatibacter actinomycetemcomitans* (Aa) en muestras biológicas de mujeres que dieron a luz a término con un diagnóstico de gingivitis y sin enfermedad periodontal. Se realizaron exámenes clínicos orales en 48 mujeres posparto, incluidas 28 mujeres con periodontitis sana, 15 con gingivitis y 5 con periodontitis (que fueron excluidas del análisis). Se recolectaron sangre de cordón umbilical (CU), sangre periférica (SP), saliva (SA) y calostro (C) de mujeres elegibles después del parto. Se realizó extracción de ADN y luego se detectó la presencia de bacterias mediante PCR con cebadores específicos. Los datos sobre embarazos anteriores y actuales y factores de riesgo conocidos se obtuvieron de los registros médicos de las pacientes. Los datos sociales, demográficos y de higiene bucal se analizaron mediante entrevista. Los resultados mostraron que todos los EA de los participantes con gingivitis tenían Pg y Aa, mientras que una minoría de muestras con periodonto sano tenían tales bacterias (P <0,05 para cada una). El 60% de las mujeres con gingivitis tenían Pg en las muestras de sangre (CU y SP, p <0,05) y el 46,6% la tenían en C. Aa no aparecía en las muestras de sangre, pero el 46,6% de las mujeres con gingivitis tenían Aa en C. sin asociación entre datos socioeconómicos y de salud bucal, detección de microorganismos y presencia de la enfermedad. En conclusión, la gingivitis se relacionó con la detección positiva de ambas bacterias en SA. Las mujeres con gingivitis a menudo tenían Pg en sus muestras de sangre. La presencia de Pg y Aa en C solo se presentó en mujeres embarazadas con gingivitis.
Palabras clave: *Porphyromonas gingivalis; Aggregatibacter actinomycetemcomitans*; Cordón umbilical sangre; Saliva; Colostrum.

1. Introduction

Molecular techniques to analyses the human microbiome acquisition (Perez-Munoz, et al., 2008) have refuted the concepts of "sterile womb paradigm" which microbes are acquired both from the mother and other humans or the environment during and after birth canal during delivery (Mackie, et al., 1999). Several microorganisms, such as *Mycoplasma hominis* (Nguyen, et al., 2004), *Enterobacter*, *Escherichia*, *Shigella* (Friedman & Jordan, 1989), *Enterococcus faecium*, *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Streptococcus sanguinis* (Jimenez, et al., 2005) had been detected in the placenta, umbilical cord blood, amniotic fluid, and meconium in term birth without any signal of infection (Aagaard, et al., 2014; Stout, et al., 2013). These findings have led many scientists proving that the "in utero colonization hypothesis" is correct, an idea that would fundamentally change our understanding of gut microbiota acquisition and its role in human development (Perez-Munoz, et al., 2008).

Study with sequencing of humans' placentas showed that their microbiomes were more like normal oral microbiota, especially the tongue and tonsil, than the vaginal or intestinal microorganisms, as previously described (Aagaard, et al., 2014). *Streptococcus mutans* was detected in mother with healthy periodontium and without caries in umbilical cord blood (Mendes,

et al, 2018) and colostrum samples. Those finds opens a range of discussions on the transfer mechanism of oral microorganisms detectable between mother and fetus, which could contribute to installation of these bacteria in the newborn or even stimulate an immune response against its installation.

Periodontal diseases (PD) are a group of oral infectious diseases caused by predominantly Gram-negative, anaerobic, and microaerophilic bacteria that colonize the subgingival area, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (Lovegrove, 2004). Gingivitis and periodontitis are a common disease during the gestational period, have occurrence around 10–20% in the general pregnancy population (Papapanou, 1996). Clinical study has reported a transient increase in the incidence and severity of gingival inflammation during pregnancy (Figuero, et al., 2010). The hormone effects are the main theories to describe this pregnancy repercussion on gingival tissue in the pregnancy on the subgingival biofilm, the immune system, the vasculature, and the specific cells of the periodontium can lead to gingival hyperplasia, gingivitis, and pyogenic granuloma (Gungu, et al., 2005; Ramos, et al., 2016).

Gingival inflammatory changes, as vasodilation, usually begin during the 2nd month and the severity increases until the 8th month of gestation (Henry, et al., 2006; Torgerson, et al., 2006), which may lead to the dissemination of microorganisms related to the periodontal disease of the mother to the fetus (Torgerson, et al., 2006). Significant differences in proportions were found for *Aggregatibacter actinomycetemcomitans, P. gingivalis, Prevotella intermedia, Tannerella forsythia, Parvimonas micra, Campylobacter rectus* and *Fusobacterium nucleatum* when comparing pregnancy to 3 months postpartum (Carrillo-de-Albornoz, et al., 2012). *P. gingivalis* challenge to the gingival tissues appears to affect the level of gingival inflammation observed during pregnancy (Carrillo-de-Albornoz, et al., 2012).

Several studies described the association between prematurity and/or low birth weight and periodontal disease (Gomes-Filho, et al., 2007; Jeffcoat, et al., 2001; Lopez, 2005; Mitchell-Lewis, et al., 2001). This association was consistent using at more than one site with clinical attachment loss (CAL) ≥ 3mm (Gomes-Filho, et al., 2007). The prematurity can occur because there are a translocation of bacteria or bacterial products from the subgingival biofilm into the systemic circulation that can reach the placenta membranes in a hematogenous form and provide the inflammatory effect to induce preterm labor (Lopez, et al., 2005). *Streptococcus* spp. and *F. nucleatum* (Bearfield, et al., 2002) and *P. gingivalis* (Blanc, et al., 2015; Vanterpool et al., 2016) were detected in the amniotic fluid in complications pregnancies. So, is clear the evidence of transference of periodontopathogens in periodontitis.

On the other hand, there are necessity to evaluate the presence of periodontopathogens, as *P. gingivalis* and *A. actinomycetemcomitans*, in human secretion of puerperal women in health condition and in diagnosed with gingivitis, and who presented with gestations without intercurrences and term deliveries. The strong evidence of microbial inflow between mother and child raises several discussions about the transfer of detectable commensal microorganisms from the mother to the fetus, such as microorganisms of the oral cavity, and their role on the stimulation of the neonate.

To aim of this study were to verify and compare the presence of Pg and Aa peripheral and umbilical cord blood, colostrum, and saliva in women with gingivitis and with healthy periodontium and data collected of general health and oral habits.

2. Methodology

Study design

The study was approved by the Ethics Committee of the Ribeirao Preto Medical School (protocol number 13290/2010) and was carried out in accordance with the principles of the Declaration of Helsinki. Informed written consent was obtained

from all patients. All volunteers were examined at the Clinical Hospital of Ribeirao Preto, Sao Paulo, Brazil. Women were orally examined until 10 hours postpartum.

Women aged 18 to 38 years old, with a singleton pregnancy, in good general health, and with a minimum of 20 permanent teeth were invited to participate. Exclusion criteria included chronic disease (i.e., diabetes, hypertension, epilepsy, cardiac disease, lung disease, renal disease, or a positive test for human immunodeficiency virus), smoking, alcohol dependency, use of systemic antibiotics, and/or psychotropic or anticonvulsant medication in the preceding 3 months. These eligible pregnant women were interviewed and signed the Informed Consent Form. Data were gathered on the pregnant women.

All women were asked to complete a self-reported questionnaire to assess their socioeconomic status (age, education level and profession) and dental care awareness (frequency of tooth brushing, last visit to the dentist and self-evaluation of their oral status). No other interventions were performed. After all evaluations and sampling procedures patients received oral hygiene instructions and received a professional prophylaxis or treatment.

Clinical periodontal examination

The clinical periodontal examination was performed in six sites per tooth except for the third molars by North Carolina periodontal probe (PCP-UNC 15, Hu-Friedy Manufacturing Inc., Chicago, USA) (Lopez, et al., 2002). Data were recorded according to the following clinical parameters: bleeding on probing (BOP), presence of calculus (PC), probing depth (PD), and clinical attachment level (AL). The highest scores of PD, CAL, BOP, and PC were registered for each tooth. Periodontal and oral examinations were conducted by a single, trained, and calibrated dentist (RDN). Cohen's kappa coefficients for intra-examiner agreement were 0.89 for PD and 0.93 for CAL.

Criteria for Periodontal Diagnosis

The presence of 4 or more teeth showing one or more sites with PD $\geq$ 4 mm and CAL $\geq$ 2 mm at the same site was diagnosed as periodontitis. All the women who did not fulfill all the criteria for periodontal disease showed gingival redness and BOP at more than 20% of sites and were diagnosed as having gingivitis (Person, et al., 2008).

Collection of samples

Peripheral blood (PB) and non-stimulated saliva (SA) were collected at the patient's admission. The saliva (SA) was collected by suction with graduated sterile pipettes and deposited in tubes and stored on ice. Umbilical cord blood (CB) samples were taken immediately after cord sectioning and deconditioning, where upon the placenta was placed on a cast support about 30 cm high. After umbilical cord antisepsis, the umbilical vein was punctured with flexible scalp and blood was collected in 50 mL syringes containing anticoagulant. Colostrum collections (C) were performed after delivery before the first feeding by manual milking after asepsis of the breasts.

Detection of microorganisms

The DNA from the studied species was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (MO-BIO, Carlsbad, CA) according to the manufacturer's instructions. Samples were transferred to tube containing Bead Solution. After were vortexed for 2 minutes to ensure the release of bacteria to the suspension, which was then transferred to a Powerlyzer Glass Bead Tube (MO-BIO, Carlsbad, CA). The concentration of the purified DNA product was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific). Primers (synthesized by Invitrogen, Carlsbad, CA, USA) and probes (synthesized by Applied Biosystems, Carlsbad, CA, USA) were targeted against 16S rRNA genes for both bacteria as described by Sanchez et al (2013)[28]: *A. actinomycetemcomitans* forward (F): 5'-GAA CCT TAC CTA CTC TTG ACA TCC GAA-3'; reverse (R): 5'

-TGC AGC ACC TGT CTC AAA GC-3', probe: 5'-AGA ACT CAG AGA TGG GTT TGT GCC TTA GGG-3'; Amplicon size: 80 bp and *P. gingivalis* (F): 5'-GCG CTCA ACG TTC AGC C-3'. (R): 5'-CAC GAA TTC CGC CTG C-3', Probe: 5'-CAC TGA ACT CAA GCC CGG CAG TTT CAA-3'; Amplicon size: 67 bp. The StepOne™ Real-Time PCR System (Thermo Fisher Scientific) performed the samples. Each reaction tube contained reaction mixture, including 6.5 µL SYBR Green Master Mix (Roche, Ilhois, EUA), 1µL of each primer, 4.5 µL de ultrapure water e 2µL of DNA extracted from samples. The cycling conditions were an initial amplification cycle of 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Extraction of DNA of both bacteria, *P. gingivalis* train ATCC 33277 and *A. actinomycetemcomitans* strain ATCC 33384, was obtained from 10^9 CFU/mL suspensions. The negative control used was sterile water. Two PCR reactions were performed for all samples.

Statistical analysis

The frequencies of samples with positivity of Aa and Pg were compared to each other and to the data obtained by the questionnaire using the Chi-square test or the Fisher's Exact Test. The differences between the numerical variables (monthly income, weeks of gestation, number of brushings, ages, intensity of fluorescence emitted in CT) were compared among the groups of pregnant women by the ANOVA test. The associations between qualitative and quantitative data were also performed by the Bisserial Point Correlation test in which the nominal variables were designated as numerical scores. A value of $p < 0.05$ was considered statistically significant. Data were analyzed using BioStat® Software.

3. Results

The study population consisted of 68 women with a mean age of 23.4 ± 5.4 years (range: 18 to 38 years). The population was ethnically diverse (55,8% white and 44.2% brown/black). Ten refused to participate the study and 10 did not have collection of all samples. Of 48 women that can participate of study, periodontitis was diagnosed in 5 women. Those women with periodontitis were excluded of study. If only women with no evidence of periodontitis (N = 43) were considered, a diagnosis of established gingivitis was exhibited by 34.9% (N = 15) of the patients. The healthy group included 28 volunteers.

Most women had a low level of education, and all were subjected to cesarean section. The mean gestational age was 39.4 ± 1.8 weeks. Nineteen regularly attended the dentist, 16 of whom claimed to have had dental follow-up during the gestational period. The average number of toothbrushes for one day was 2.7 ± 0.8 times. There were no associations between groups of women with gingivitis and healthy, regarding race, age, education, gestational age ($p>0.05$). Data collected on oral health and hygiene habits were not associated between groups (Table 1, $p> 0.05$).

The distributions of bacteria that differed between volunteers with or without a diagnosis of gingivitis are shown in Table 2. *P. gingivalis* was detected in all salivary samples of patients with gingivitis and associated with the presence of oral disease (Tab. 2, $p<0.001$). Among the healthy women, 32.1% presented Pg in the saliva, but none presented this bacterium in the blood and colostrum samples (Table 2). The frequencies of positive samples for Pg showed that of the women with gingivitis, 60% presented of Pg in the PB and CB, and 46.6% transferred to the C. The presence of Pg in the blood and colostrum were associated with the presence of oral disease (Tab. 2, $p<0.001$).

Analyzes of Aa detection showed that this bacterium was found in all SA samples of patients with gingivitis and was associated to disease (Table 2, $p<0.001$). Seven gingivitis women (46.6%) presented Aa in C (being the same mothers who presented Pg) and none in the blood samples (Table 2). Among samples of periodontal healthy, 32.1% had Aa in detectable levels, only in the SA samples (Table 2). The comparative analysis between the microbial strains represented in the Figure 1 shows that the positive detection of both Pg and Aa occurred in the same samples of SA and C of gingivitis and healthy women.

The intensity of fluorescence emitted in CT for positive detection of Pg or Aa showed that there were no statistically significant differences (p>0.05) in the intensity in the samples and groups (p>0.05)

There was no association between the data collected in the questionnaire and presence or absence of two bacteria in the samples analyzed (race/ethnicity, visits to the dentist, dental treatment during gestation, number of brushings during the day) (p>0.05).

Table 1. Frequency and percentage of women with gingivitis and healthy according to number of teeth brushing in one day, preventive dental treatment (included dental sealant, dental scaling, and fluoride application) and dental visit experience in a year.

Variables	**Gingivitis N=15 n (%)**	**Healthy N=28 n (%)**	**Total N=43 n (%)**	***P*-value**
Frequency of tooth brushing				
≥3 times/d	9 (60.0)	16 (57.1)	25 (58.1)	0.89
<3 times/d	6 (40.0)	12 (42.9)	18 (41.8)	
Preventive dental treatment *(included dental selant, dental scaling and fluoride application)*				
Yes	4 (26.7)	12 (42.9)	16 (37.2)	0.34
No	11 (73.3)	16 (57.1)	27 (62.8)	
Dental visit experience in a year				
Yes	6 (40.0)	13 (46.4)	19 (44.2)	0.93
No	9 (60.0)	15 (53.6)	24 (55.8)	

Source: Authors.

Table 2. Detection of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* in samples of saliva (SA), peripheral blood (PB), umbilical cord blood (CB) and colostrum (C) of women with gingivitis and healthy.

	P. gingivalis				*A. actinomycetemcomitans*			
Detection of bacteria in the samples of:	**Gingivitis N=15 n (%)**	**Healthy N=28 n (%)**	**Total N=43 n (%)**	***p***	**Gingivitis N=15 n (%)**	**Healthy N=28 n (%)**	**Total N=43 n (%)**	***p***
SA								
Yes	15 (100.0)	9 (32.1)	24 (55.8)	<.0001	15 (100.0)	9 (32.1)	24 (55.8)	<.0001
No	0 (0.0)	19 (67.8)	19 (44.2)		0 (0.0)	19 (67.8)	19 (44.2)	
PB								
Yes	9 (60.0)	0 (0.0)	9 (20.9)	<.0001	0 (0.0)	0 (0.0)	0 (0.0)	1.000
No	6 (40.0)	28 (100.0)	32 (79.1)		15 (100.0)	28 (100.0)	32 (100.0)	
CB								
Yes	9 (60.0)	0 (0.0)	9 (20.9)	<.0001	0 (0.0)	0 (0.0)	0 (0.0)	1.000
No	6 (40.0)	28 (100.0)	32 (79.1)		15 (100.0)	28 (100.0)	32 (100.0)	
C								
Yes	7 (46.7)	0 (0.0)	25 (58.1)	<.0001	7 (46.7)	0 (0.0)	7 (16.3)	<.0001
No	8 (53.3)	28 (100.0)	18 (41.9)		8 (53.3)	28 (100.0)	36 (83.7)	

Source: Authors.

4. Discussion

The present study sought to analyze the presence of Pg and Aa in samples of peripheral blood, maternal colostrum and saliva, and umbilical cord blood from women with gingivitis or healthy periodontium and who did not present any complications during pregnancy and to compare the data obtained with socioeconomic information and oral health.

The choice of species occurred because bacteria were frequently associated with periodontal disease (Borgo, et al., 2014). Although the detection did not differentiate living microorganisms, dead or bacterial fragments, the assays were efficient for DNA detecting of Pg and Aa in the standard strains, as well as in the samples tested. The use of PCR was previously tested and effective practice for the detection of these bacterial species (Periasamy & Kolenbrander, 2009). In addition, the primers used were tested by Yoshida et al. (2003) in salivary samples and are highly specific for species detection.

Samples were obtained from women with low income and educational level who did not present intercurrences during pregnancy and did not have low birth weight children. Cruz and coworkers (2005) established a strong association between the presence of gingivitis and low socioeconomic conditions and difficulty in accessing health services, as well as health-related behaviors such as smoking, alcoholism, a high carbohydrate diet and poor oral hygiene. The results obtained here did not allow establishing such associations between the diagnosis of the disease and socioeconomic and behavioral data.

The percentage of periodontitis found (10.4%) was different from the results of Kunnen and coworkers (2007), who found the disease in 37% of pregnant women. However, Lima and coworkers (2014) showed the same percentage, in which 10.5% of pregnant women presented with periodontitis.

The results showed that all salivary samples from patients with gingivitis presented Pg and Aa, as found in previous studies (Casarin, et al., 2010, Deng et al., 2017; Liu, et al., 2013). Gingivitis in the gestational period is a common situation due to changes in vascular permeability, in the immune system and in the subgingival biofilm (Raber-Durlacher, et al., 1994).

Hormonal variation may promote the overgrowth of pathogenic bacteria responsible for gingival inflammation (Mascarenhas, et al., 2003). Although *P. gingivalis* is not considered a normal inhabitant of a periodontal healthy dentition (Griffen, et al., 1998) and is rarely found in healthy gums (Amano, et al., 2000), here, 9 periodontal healthy women (32.1%) had detectable levels of Pg and, Aa in the salivary samples.

Many women with gingivitis had Pg in PB (60%). The presence those bacteria, in the maternal peripheral circulation in patients with periodontal disease, are well described. Transient bacteremia can happen even after brushing in patients with gingivitis (Marin et al., 1998). Subgingival biofilm is close to the inflamed tissues around which allows entry of bacteria in the blood and dissemination to distant organs (Herzberg & Weyer, 1998). In addition, gingival increased permeability during pregnancy further facilitates the entry of bacteria into the blood, which could be transferred into the fetal circulation.

The presence of oral bacteria in the placenta, associated with periodontal disease, such as *Fusobacterium nucleatum*, *Streptococcus* spp were described in women who had premature births (Bearfield, et al., 2002; Vanterpool, et al., 2016). *Porphyromonas gingivalis* and its involvement with premature birth seems to depend on the colonization of the microorganism, that must be in the stroma of the placental and in the umbilical cord tissues of pregnant women[25]. In term pregnancies did not find Pg at these sites (Vanterpool, et al., 2016). Our results showed evidence of the presence of Pg in 60% of cord blood samples analyzed cannot have to lead the premature birth because it was not present in the placental tissues and also was insufficient to stimulate a local inflammatory response, as occurs mainly in periodontitis and has a high correlation with preterm births (Offenbacker, et al., 1998).

The presence of Pg in CB of pregnant women with gingivitis, corroborates the findings of recent studies, in which several microorganisms could be isolated in samples of umbilical cord blood, amniotic fluid, and in the placenta with no clinical or histological evidence of infection or inflammation in the mother-child pairs (Jimenez, et al., 2005; Stout, et al., 2013) and refute the idea that the intrauterine environment is free of microorganisms and that fetuses acquire microorganisms only when they started transit through the vaginal canal and subsequently through contact with maternal skin (Mackie, et al., 1999). Metagenomic analysis of placental specimens has revealed the existence of a microbiome in the placenta with taxonomic profiles like those described in the oral environment, where periodontopathogen bacteria are relatively abundant in placentas of healthy pregnancies (Aagaard, et al., 2014, Prince, et al., 2014). Our results are consistent with these facts. Although Blanc and coworkers (2015) did not find Aa and Pg, the authors found oral bacteria such as *A. israelii* and *F. nucleatum* in more than 27% of the placental specimens of full-term mothers without periodontal disease. Thus, the presence of Pg in CB in full-term pregnancies allows us to accept the asymptomatic carrier state (Katz, et al., 2006). Complications during gestation may be the result of changes in the placental microbiota and in the number of micro-organisms, i.e, the levels of oral pathogens in the placenta depend on the periodontal status of the mother (Blanc, et al., 2015). Colostrum of 46% pregnant women with gingivitis presented Pg and Aa. The pathway of entry of bacteria into the mammary glands and human milk is still unclear.

The pathways of entry of bacteria into the mammary glands and its exit by colostrum and mature milk are still unclear. There are some hypotheses to explain the presence of bacteria in the mammary glands, firstly due to the contact with external environment, or by paths within the human body or by an association between both pathways (Jost, et al., 2015). External contamination can occur through contact and transfer of the neonatal buccal and skin microbiota during breastfeeding (Ramsay & Hartmann, 2005) that can explain the predominance of in the human milk, of *Staphylococcus* and *Streptococcus*, typical commensals of oral and skin microbiota (Kong & Segre, 2012). However, the collection of the colostrum was performed before the first contact with neonatal and after the asepsis of breasts. Another pathway involves an internal bacterial route that occurs when oral microorganisms reach the maternal intestine and leaves by internalization in leukocytes (such as dendritic cells) and migrates to the lymphatic pathway, blood circulation, and reaches the mammary glands (Jost, et al., 2014; Martin-Sosa, et al., 2004) justifying the presence of Pg and Aa in part of samples.

5. Conclusion

The present results reinforce the theory that there are bacteria or part of them circulating in the peripheral blood and umbilical cord blood, without causing an early interruption of gestation. This study opens a range of discussions on the transfer mechanism of oral microorganisms detectable between mother and fetus, which could contribute to installation of these bacteria in the newborn or even stimulate an immune response against its installation. Both bacteria were detected in a few samples of colostrum. Most women with gingivitis presented Pg in blood samples, which did not happen for Aa. There was no association between the diagnosis of the disease, presence of the microorganism and socioeconomic and behavioral data.

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