Identification of Microorganism Crops in Dental Plaque of Pregnant Women

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Abstract

Alterations in the oral microbial community during pregnancy may affect maternal oral health, birth outcomes, and infant oral health. Methods: Cross-sectional observational study. The patients included in this study; pregnant women who were in the first trimester of pregnancy, a survey was applied to them. For the review of periodontal health, it was evaluated with the Russell periodontal index. The supragingival plaque samples were collected in 13x100 test tubes with screw caps, containing 3 ml of Brain-Heart Infusion (BHI), the plaque samples infragingival were collected in tubes of the same size, with Fluid Thioglycollate Medium (FTM). The samples were sent to the Epidemiology Laboratory of the ESCN, Guerrero, and were processed within a maximum period of 24 h. cultures were carried out under aerobic and anaerobic conditions, identified by microscopy, Gram staining and biochemical tests. Demographic data and clinical variables were analyzed using the Student's t-test, the ANOVA test, and the chi-square test with probability values adjusted with Bonferroni correction.

Results: A total of 60 samples were recovered from the 10 patients, 30 subgingival plaque and 30 infra gingival plaque, taken in the three trimesters of pregnancy. Of the 60 samples analyzed, 194 strains were obtained (100 supragingival and 94 infra gingival), of which 41.45% (80) were gran-positive coccus, 19.68% (39) gran-negative coccus, 12.43% (24) gram positive bacilli, 11.43% (22) gram negative bacilli, 4.66% (9) gram positive micrococcus, 5.69% (11) gram negative diplococcus and 4.66% (9) yeasts.

Conclusion: The dental plaque in our participants is composed of Gram-positive coccus and bacilli (53.88%), Gram-negative coccus and bacilli (31.01%), micrococcus (4.66%), diplococcus (5.69%) and yeast (4,66%).

Introduction

The oral cavity is colonized with a complex and diverse microbiome of more than 700 commensals that have been identified in the Human Oral Microbiome Database, including bacterial and fungal species. Since a balanced microbial flora helps maintain stable oral and general health, disturbances in the oral microbial community during pregnancy can affect maternal oral health, birth outcomes, and infant oral health [1].

During pregnancy, many changes occur in the oral cavity that may be related to periodontal disease, including gingivitis and periodontitis. The oral health of pregnant women can worsen due to hormones or changes in diet and hygiene [2]. Approximately 60% to 75% of pregnant women have gingivitis [3].

Periodontal diseases are accentuated due to hormonal changes: higher rates of gingival inflammation have been observed in pregnant women compared to non-pregnant women. In addition, some studies have shown a high prevalence of dental caries among pregnant women [2]. Bacteria like *Streptococcus mutans* are the main etiological agent of human dental caries [4]. Behavioral changes that can occur during pregnancy, such as difficulties performing good oral hygiene and increased sugar intake, lead to higher plaque scores [2].

Epidemiological and clinical studies suggest that maternal periodontitis may be associated with an increased risk of adverse pregnancy outcomes, such as low birth weight, preterm delivery, miscarriage, and/or stillbirth [5]. Preterm birth and preeclampsia are known to be associated with maternal inflammatory complications. Several studies have reported the association of periodontal diseases with preterm birth and preeclampsia [4]. Therefore, it is essential to understand the changes in the oral flora during pregnancy, as well as to identify the microorganisms found in the oral cavity in pregnant women according to their stage of gestation.

Materials and methods

Study design

This study was designed as a cross-sectional observational study.

Patient sample

The patients who attended the community hospital of the municipality of Cópala, Gro. were randomly selected. Their data such as sociodemographic profile, age, smoking habit, systemic health, and medications, among others, were recorded. The patients included in this study met the following characteristics: pregnant women who were in the first trimester of pregnancy. When the patients met this criterion, they were verbally informed about the study and asked to participate by signing an informed consent. After acceptance, each patient was given a survey.

Clinical examination

To assess the Russell periodontal index, all teeth were examined in detail, considering the presence of gingivitis and its severity, tooth mobility, presence/absence of periodontal pockets, bone destruction, etc. Periodontal tissues were evaluated separately for each tooth. The following formula was used to calculate the index: Russell index = total sum of the evaluation of each tooth/total number of teeth of the patients. With a Russell index value of 0 to 1.4, the patient was considered to have an initial or mild periodontal lesion, a value between 1.5 and 4.0 indicates a medium periodontal lesion, and 4.0 to 8. .0 is a sign of a severe periodontal disorder.

Microbiological procedures

Microbiological sampling

Supragingival plaque samples were collected in 13x100 screw-cap test tubes containing 3 ml of Brain Heart Infusion (BHI), infra gingival plaque samples were collected in tubes of the same size, with Fluid Thioglycollate Medium (FTM), once the samples were obtained, they were transported in racks inside a thermos with frozen refrigerant. The samples were sent directly to the Epidemiology Laboratory of the ESCN, Guerrero, where they were processed within 24 h.

Direct anaerobic crop

Infragingival plaque samples were inoculated onto nutrient agar, MacConkey, Biggy, and non-selective blood agar medium, supplemented with 5% sterile sheep blood. The plates were incubated for 48 to 72 hours under anaerobic conditions (80 % N_2, 10 % CO_2 and 10 % H_2) at 37 °C. After 72 hours of anaerobic incubation, suspicious colonies were identified by microscopy, Gram stain, and biochemical tests, according to Representative colony counts were carried out.

Isolation of pathogens from supragingival plaque was performed using the aforementioned agar media, by depletion streaking, which was incubated for 2 days in the air with 5% CO2 at 37°C.

Statistic analysis

Demographic data and clinical variables were analyzed using the Student's t-test, the ANOVA test, and the chi-square test with adjusted probability values with Bonferroni correction. For microbiological outcome variables, total anaerobe counts were calculated on blood agar plates and expressed in total colony-forming units/ml (CFU/ml). Counts of each specific bacterial species, as well as their percentage of total culturable bacteria, were also calculated for each patient. All statistical analyzes were performed with the SPSS 20 software package, and the values of $p< 0.05$ were considered significant.

Results

In this study, 10 patients participated, from which a total of 60 samples were recovered, 30 subgingival plaque and 30 infra gingival plaque, taken in the three trimesters of pregnancy.

During the follow-up of the patients during the pregnancy period, each of the pregnant women stated that they had had some discomfort and it was found that 90% of them presented caries in most of their teeth. 50% of the pregnant women suffered from gingival disease, and they also stated that they had inflammation and bleeding in their gums, which was confirmed at the time of the periodontal assessment. In addition, 10% of the study population mentioned the appearance of a granuloma gravidarum in some parts of the gum and also cold sores **(Table 1)**.

Continuing with the exploration of dental health data, 40% had bleeding during tooth brushing. Of the population studied, 40% had healthy gums while 60% presented gingivitis. On the other hand, 60% presented threats and urinary tract infections **(Table 1)**.

60 dental plaque samples were obtained, which were taken quarterly from 10 pregnant women, 30 from the supragingival region and 30 infra gingival, from which a total of 194 bacterial strains were obtained (100 supragingival and 94 infra gingival).

Of the 60 samples analyzed, 194 strains were obtained (100 supragingival and 94 infra gingival), of which 41.45% (80) were gram-positive coccus, 19.68% (39) gram-negative coccus, 12.43% (24) gran-positive bacilli, 11.43% (22) gram-negative bacilli, 4.66% (9) gram positive micrococcus, 5.69% (11) gram-negative diplococcus and 4.66% (9) yeasts **(Figure 1)**.
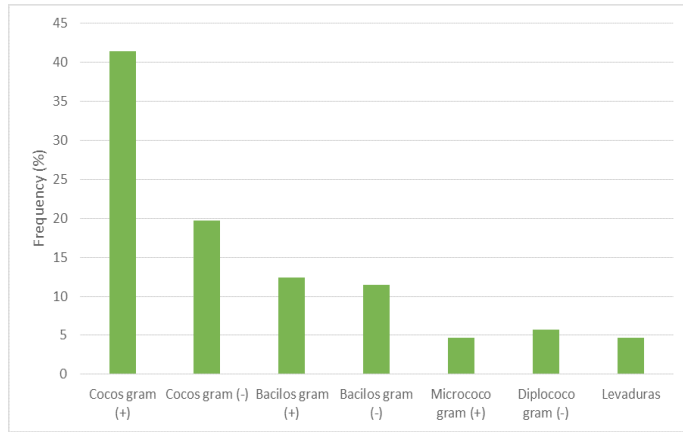

Figure 1: Frequency of strains isolated from dental plaque of pregnant women. You can observe that gran coccus (+) were those that predominated in the three trimesters of pregnancy with 41.45%.

When analyzing the isolated strains according to the trimester of pregnancy, the gender that predominated in the three trimesters was *Streptoccoccus mutans* with 21.64% (42) and *Veillonella spp,* with 17.52% (34). During the first and third trimesters, the highest number of strains isolated from dental plaque was *Streptococcus mutans* with 22.38% and 25.49%, respectively. While in the second quarter, the gender predominated was *Veillonella spp.* with 22.36% **(Figure 2)**.

Interestingly, there were genera that only appeared in some trimesters of pregnancy, for example, *Candida* spp., Streptococcus *mitis,* and *Streptobacillus* which were only present in the second and first trimesters. *Lactobacillus spp present only in the second and third trimesters of pregnancy and Prevotella spp.*

Present in the dental plaque of pregnant women in the first and third trimesters. Likewise, genres like *Streptococcus salivarius* and *Streptococcus sanguinis,* that only occurred in the first and third trimesters, respectively **(Figure 2)**.

The main pathologies caused by the identified bacteria were investigated and categorized according to the Socransky complex **(Table 2)**. Of the identified bacteria that are not part of the oral cavity (autochthonous), disk microbial susceptibility tests were performed. Finding that *Fusobacterium nucleatum* It is sensitive to Pefloxacin, Ceftriaxone, Chloramphenicol, Ceftazidime, Ciprofloxacin, and Amoxicillin. While *Salmonella* spp. only showed sensitivity towards Ciprofloxacin **(Table 3)**.
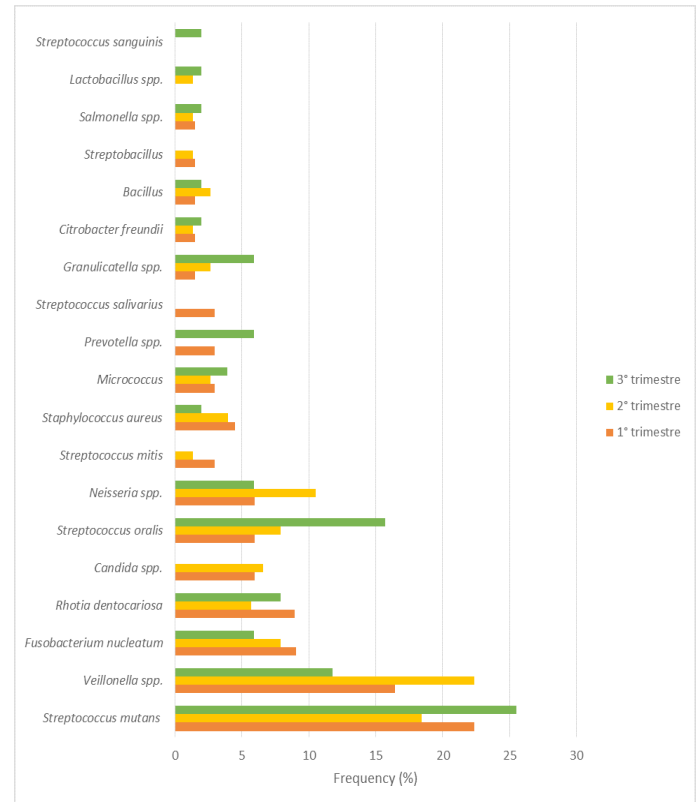

Figure 2: Frequency of microorganisms isolated from dental plaque of pregnant women during the three trimesters of pregnancy. It is observed that the predominant genres during the three quarters were *Streptococcus mutans* and *Veillonella spp*.

Table 2: Bacteria identified in dental plaque, compared in the category of Socransky complex bacteria and their associated pathologies.

	Socransky complex	Associated pathology
Streptococcus mutans	Yellow	Caries, subacute infective endocarditis, digestive system neoplasms.
Veillonella spp	Purple	Gingivitis, abscesses, tonsil,
Streptococcus oralis	Yellow	Subacute endocarditis.
Fusobacterium nucleatum	Orange	Periodontitis, endocarditis, myopericarditis, preterm birth, septic arthritis
Rothia dentocariosa	Not belonging to the complex	The development of caries can spread through the hematogenous or lymphatic route, causing infectious metastases in various organs such as: the heart, liver, and kidney.
Staphylococcus aureus	Not belonging to the complex	Boils or abscesses, acne, infectious dermatosis, cystitis, pyelonephritis, tonsillitis, rhinitis, pneumonia, paronychia, ecthyma, omphalitis
Neisseria spp	Not belonging to the complex	Nasopharyngeal infections, meningitis
Prevotella spp	Orange	Chronic periodontitis, actinomycosis, endodontitis, gingivitis, peritonitis
Streptococcus mitis	Yellow	Endocarditis, dental abscesses, brain abscesses
Streptococcus salivarius	Yellow	When found in blood circulation, it has been implicated in cases of sepsis

Streptococcus sanguinis	Yellow	Caries, Endocarditis, dental abscesses, and abscesses in deep tissues. Lung abscesses, thoracic empyema, meningitis
Lactobacillus spp	Yellow	Advanced caries
Streptobacilos spp	Yellow	Pharyngotonsillitis, pyoderma, erysipelas, scarlet fever, rheumatic fever, impetigo, rhinitis pneumonia, glomerulonephritis, meningitis
Streptococcus viridans	Yellow	Meningitis, septicemia, caries, endocarditis, abscesses, post craniotomy
Salmonella	Not indigenous to the oral microbiome	Typhoid fever, Diarrhea, low immune response, gastrointestinal tract infection
Citrobacter freundii	Not indigenous to the oral microbiome	Respiratory infections, pneumonia, meningitis, nosocomial sinusitis
Candida spp	Yeast	Thrush, candidiasis

Table 3: Antibiogram for non-native bacteria isolated in dental plaque from pregnant women.

Microorganism	Antibiotic					
Salmonella spp	Pefloxacin	R	Carbenicillin	R	Ceftazidime	R
	Cefazolin	R	Cefotaxime	R	Ciprofloxacin	S
	Nitrofurantoin	R	Netilmicin	R	Clindamycin	R
	Ceftriaxone	R	Gentamicin	R	Amoxicillin	R
	Chloramphenicol	R	Amikacin	R	Ampicillin	R
	Penicillin	R				
Fusobacterium nucleatum	Pefloxacin	S	Carbenicillin	R	Ceftazidime	S
	Cefazolin	R	Cefotaxime	R	Ciprofloxacin	S
	Nitrofurantoin	R	Netilmicin	R	clindamycin	R
	ceftriaxone	S	Gentamicin	R	amoxicillin	S
	Chloramphenicol	S	Amikacin	R	Ampicillin	R
	Penicillin	R				

(R: Resistant S: Sensitive SI: Intermediate Sensitivity) CLSI Standards juanuary 2015.

Discussion

In the 60 samples of dental plaque in pregnant women, a total of 194 strains were isolated, which correspond to the three samples obtained in each trimester of pregnancy. During the first and third trimesters, the highest number of strains isolated from dental plaque was *Streptococcus mutans*. While in the second quarter, the predominated gender was *Veillonella spp.*

In the first trimester, the bacteria of the yellow complex were found, which are the usual ones in the oral cavity, such as *S. mutans, S. oralis, S. viridans, and S. mitis*. In the second trimester, *Fusobacterium nucleatum* belonging to the orange complex was found.

In this study, the strains of *Streptococcus mutans* represented the highest percentage of isolates 21.64%. Similar data were described by [6], who found that S. mutans predominated in the saliva of pregnant women, particularly during the first trimester, as in this study. *Veillonella spp*. it was the second most abundant genus in the dental plaque of pregnant women. This bacterium has been found frequently in pregnant women with gingivitis [7].

Fusobacterium nucleatum was the third genus that occurred frequently in the three trimesters of pregnancy. *F. nucleatum* is a Gram-negative filamentous spindle bacillus that is a common inhabitant of the oral flora. There is speculation that it may act as an opportunistic pathogen in relation to extraoral sites, as it has been implicated in diseases such as appendicitis, brain abscesses, and chorioamnionitis [8]. *F. nucleatum* has evolved in close association not only with mammalian cells and tissues found in the oral cavity but also with the oral microbiota. *F. nucleatum* plays integral and beneficial roles in biofilms that contribute to both periodontal health and disease. In a dental plaque biofilm, *F. nucleatum* fulfills a structural support function as a bridging organism, connecting primary colonizers such as species of *Streptococcus* with the largely anaerobic secondary colonizers to which it can also bind, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans included* [9]. Clinical studies have linked *F. nucleatum* with various pregnancy complications, including preterm delivery, stillbirth, and neonatal sepsis [5,1].

In addition, bacteria of the genus *Salmonella spp,* that is not part of the normal microbiota of the oral cavity. Due to the involvement of this bacterium in human health, it is necessary to highlight that it is currently known that the hematological dissemination of oral microorganisms and their products would subsequently induce an inflammatory/immune response in the fetoplacental unit, being detrimental to the product and mother [10].

Yeasts associated with pregnancy were also found, *Cándida spp* identified in the first and second trimester of pregnancy which causes candidiasis in pregnant women which can be transmitted from mother to infant, exchange of mother and child oral fluids, kiss on the mouth (appearance of milkweed).

Mentions that bacteria can be lodged in many areas of the mouth apart from the teeth, such as the tongue and gums. The use of mouthwash and dental floss after brushing is important

to reduce their proliferation, since brushing does not reach some areas of the teeth, the use of mouthwash helps to reduce these microorganisms, in our study, only 10% used mouthwash while 30% used dental floss. In our study population, none of the pregnant women used any cleaning treatment other than brushing during their pregnancy, which led to favorable conditions for the appearance of certain bacterial strains, such as *salmonella spp*, *Fosubacterium nucleatum*, *Citrobacter freundii* that are not native to the oral cavity.

Mentions that people who consume a lot of foods or drinks rich in sugars favor the development of bacterial species that ferment sugars, unbalancing the bacterial population and favoring the development of species such as *Streptococcus mutans* and *Lactobacillus spp*, that produce acids that dissolve the protective enamel of the teeth, which leads to the consequences of two most prevalent infections, being caries and periodontitis, compared to our study the most prevalent microorganism was *Streptococcus mutans* Likewise, the most prevalent infection identified in this study was caries in pregnant women.

Conclusions

The dental plaque in our participants is composed of Gram-positive coccus and bacilli (53.88%), Gram-negative coccus and bacilli (31.01%), micrococcus (4.66%), diplococcus (5.69%) and yeast (4,66%).

A total of 194 strains were isolated, identifying 19 genera, most frequently the genus of *Streptococcus mutans* predominating more in the first trimester, in the second trimester with greater frequency the gender of *Veillonella spp*, and in the third trimester the gender *Streptococcus mutans*.

The oral disease that occurred most frequently in pregnant women is caries, one of the most common oral diseases worldwide.

The clinical and statistical analyzes showed that the population in this study did not practice brushing or flossing correctly and did not use mouthwash since they are one of the important factors for the development of dental plaque.

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