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Relationship between dental caries experience and the levels of Streptococcus mutans and lactobacillus in saliva of pregnant women

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ABSTRACT

Pregnancy-related changes may increase the risk of dental caries. The Cariogram software program was created to understand this risk better. This study aims to correlate dental caries experience with Streptococcus mutans and lactobacillus levels in saliva using real-time PCR. This case-control analytical study conducted in Erbil City, Iraq, between 2021 and 2023 used a Cariogram to assess tooth decay risk and a real-time PCR assay to detect oral bacteria. Kurdish chewing gum with oil extract was used to stimulate saliva production. The chance of preventing tooth decay was 50.57% in pregnant women and 60.26% in non-pregnant women, with a statistically significant difference. The correlation between caries risk categories and Streptococcus mutans and Lactobacillus levels in saliva was significant but weakly positive, with strengths of 0.295 and 0.213, respectively. Furthermore, the proportion of pregnant women with Lactobacillus class 2 or Lactobacillus class 3 was significantly higher than that of non-pregnant women (7% and 10% versus 2% and 1%, respectively) with a p of 0.001. The study also found that 82% of pregnant women had a very low or zero amount of Streptococcus mutans compared to 96% of non-pregnant women (p = 0.011). The study concluded that Streptococcus mutans and Lactobacillus (SM and LBs) could be accurately detected through qPCR, and their counts have a significant positive correlation with caries risk categories. These bacteria are considered important causal agents of dental caries, especially in pregnant women.

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Introduction

Dental caries is a prevalent infectious disease that affects a large portion of the global population, particularly in economically disadvantaged areas (1–3). The primary cause of caries is an ecological imbalance in the oral cavity. When caries-related bacteria increase and beneficial bacteria decrease, dental plaque can transition from non-cariogenic to cariogenic (4). Dental caries is associated with acidogenic and acid-tolerating bacteria like Streptococcus mutans and lactobacilli. While S. mutans is widely recognized as the main cause of tooth decay, various species of lactobacilli contribute to lesion progression, particularly in dentin (5). Both S. mutans and lactobacilli produce acid from carbohydrate metabolism and thrive in low-pH environments (6). However, lactobacilli have poor adherence to oral tissue and are typically missing or present in small amounts in healthy mouths, but they are more prevalent in areas with active caries, especially carious dentine (7).

Klock and Krasse were the first to suggest a quantitative study of S. mutans in saliva, discovering that the number of these microorganisms in dental bacterial plaque is strongly linked to their salivary concentration (8). Knowing both S. mutans and Lactobacillus counts improves the accuracy of the microbiological evaluation, as an elevated salivary level of S. mutans is not a reliable indicator of caries risk (9,10).

Pregnant women are highly prone to tooth decay due to factors like increased mouth acidity, cravings for sweet foods, and less attention to oral hygiene (11,12). Pregnant women had 2.2 times as much dental decay and 1.94 times as much gum disease as non-pregnant women, according to a study by Swati Patil et al. (2018) (13). Specialists can prevent caries by identifying susceptible individuals and using screening approaches to identify risk factors (14). Choosing an accurate caries risk assessment technique like Cariogram® is crucial for successfully predicting and preventing tooth decay (15).

According to studies, pregnant women have greater total viable oral microbial counts throughout their pregnancies than non-pregnant women, especially in the first trimester. Additionally, it has been shown that pregnancy changes the development and proliferation of several bacterial taxa, including Lactobacillus, Bifidobacterium, Streptococcus, and Escherichia coli (16,17). Therefore, this study aimed to determine the relative amounts of Streptococcus mutans (SM) and lactobacilli (LBs) in saliva and their relationship with dental caries categories assessed by the Cariogram manual among pregnant women in Erbil, Iraq.

Materials and Methods

This was a case-control analytical study conducted in Erbil City, Iraq between 2021 and 2023. Official approval to conduct this study was obtained from the Dean of the College of Dentistry/Hawler Medical University, Erbil-

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Iraq. The sample size consisted of 200 subjects, including 100 pregnant women in their third trimester and 100 non-pregnant women. The inclusion criteria for the study were: pregnant women in their third trimester without pregnancy risk, informed consent, and aged between 20 and 40 years old. Exclusion criteria were: subjects with prostheses, fixed orthodontic appliances, antibiotic therapy, history of chemo/radiotherapy to head and neck, combination usage of tobacco, alcohol consumption, usage of mouthwash or undergone oral prophylaxis in the past 1-month, and uncooperative patients.

The study involved five steps to assess caries risk: a questionnaire, interview, clinical examination, saliva sampling, and the Cariogram. The questionnaire covered various factors, including demographics and oral hygiene habits (18,19). The clinical examination followed the WHO criteria; visible lesions with cavities were classified as dental caries; and initial enamel demineralization with intact surfaces and no cavities were considered intact teeth (20). Past caries prevalence was registered at the DMFT level. Oral hygiene and biofilm amount were estimated using the Sillness and Löe plaque index (21).

In this study, saliva samples from participants were collected for analysis. The process included measuring saliva secretion rate, and buffer capacity using J-pHix®, and detecting specific bacteria with PCR. Participants were instructed not to perform oral hygiene measures, eat, or smoke for 1 hour before collection (1,2,22–27). Kurdish chewing gum was used to stimulate saliva production, and samples were stored at -20°C until analysis (2,27,28). DNA concentration and quality were determined using the NanoDrop device, with acceptable values being a concentration above 10ng/µl and specific ratio values.

According to the results of Nanodrop during 260/280, there was both low DNA impurity and suitable concentration to perform PCR and Real time PCR (Table 1).

Real-Time PCR detection

The study used a qPCR assay with a CFX96 thermal cycler to detect oral bacteria, S. mutans, and Lactobacillus spp in genomic DNA samples. Each reaction tube contained qMAXSenTM gREENqPCR Master Mix, DNA samples, primers, and nuclease-free water. The 25 µl reaction mixture included 5 µl of DNA, 2 µl of each primer, and 3.5 µl of nuclease-free water. The thermal cycler protocol included activation for 10 min at 94°C followed by 40 cycles of denaturation, primer annealing, and extension (1,29,30). Results were represented as CFU/ml (31,32), and primers were checked and optimized by conventional PCR and evaluated by agarose gel electrophoresis (2,29,33-34). The sequences of primers used are listed in Table 2.

Using the Cariogram, a risk profile was created by assigning scores of 0-2 or 0-3 to caries-related factors. Five Cariogram categories were used: “very high risk” (0-20% chance to avoid caries); “high risk” (21-40% chance to avoid caries); “moderate risk” (41-60% chance to avoid caries); “low risk” (61-80% chance to avoid caries); and “very low risk” (81-100% chance to avoid caries) (35). Data was entered into the Cariogram to obtain an individual caries risk profile (Table 3).

Table 1. DNA purity 260/280 ratio.

260/280 Ratio	F (%)
< 1.7 protein contamination	16 (8%)
1.7 – 2 Suitable	171 (85.5%)
> 2 a lot of RNA	13 (6.5%)

Results

In this study, 200 women (100 pregnant, 100 non-pregnant) were examined. The mean age of pregnant women was 27.84 years (SD = 6.32) and for non-pregnant women was 26.54 years (SD = 5.99), but there was no significant age difference. Non-pregnant women had significantly higher rates of college education (50% vs. 25%, p = 0.003) and high-ranking occupations (17% vs. 4%). The majority of pregnant women were housewives compared to non-pregnant women (83% vs. 51%, p < 0.001). No significant differences were found between the two groups for husband's education and occupation, car ownership, and income (p = 0.462, p = 0.962, p = 1.000, p = 0.664, respectively). (Table 4)

The chance of preventing tooth decay was 50.57% in pregnant women and 60.26% in non-pregnant women. The susceptibility sector had a risk structure profile of 31.50%, while the bacteria sector had a risk structure profile of 9.02%. Pregnant women had a significantly lower chance of avoiding new caries (mean = 50.5, mean rank = 88.5) compared to non-pregnant women (mean = 60.2, mean rank = 112.4) with a p of 0.003. The mean and mean rank of circumstances, bacteria sector, and susceptibility sector parameters were significantly higher in pregnant women than in non-pregnant women (p < 0.05). However, there were no significant differences in the diet sector between the two groups with a p of 0.235. (Table 5) (Figure 1)

Table 6 shows that the percentage of caries-free in pregnant women was only 8% compared to 6% in non-pregnant women. However, no statistically significant differences were detected between the two study groups regarding categories of past caries experience (P = 0.952) and the proportions of pregnant women who ate moderate (Lactobacillus class 2) or high fermentable carbohydrate (Lactobacillus class 3) content diet (7% and 10% respectively) were significantly (P = 0.001) higher than the proportions among non-pregnant women (2% and 1% respectively). Regarding Streptococcus mutans, almost all (96%) of the non-pregnant women had a very low or zero amount of Streptococcus mutans, compared with 82% of the pre-

Table 2. Primer sequences and optimal annealing temperature.

Primer Name	Sequence 5′ to 3′	Optimal Annealing Temperature	References
Sm F5 Primer	5′- AGCCATGCGCAATCAACAGGT-3′	64 °C	**Yano et al., 2002** (34)
Sm R4 Primer	5′- CGCAACGCGAACATCTTGATCAG -3′		
Lacto F Primer	5′- TGGAAACAGRTGCTAATACCG-3′	62 °C	**Byun et al., 2004** (33)
Lacto R Primer	5′- GTCCATTGTGGAAGATTCCC-3′		

Table 3. Caries-related factors/parameters used at baseline for the Cariogram.

Factor	Information to be collected	Cariogram score
Caries experience	DMFS, new caries experience, and DMFT	0: No fillings or caries 1: Above mean for the age group 2: Standard for the age range 3. Worse than the mean for the given age group
Related general diseases	health background and medications	0: Healthy 1: Existence of a general illness that may have an indirect impact on the development of caries 2: Constantly taking medicine or being bedridden
Diet content	Diet history (and or Lactobacillus Test by qPCR): dietary quality	0=Very low (≤ 10^3 CFU/ml, very low sugar consumption) 1=Low (10^4- 10^5 CFU/ml, low sugar consumption) 2=Moderate (10^5-10^6CFU/ml, moderate sugar consumption) 3=High (≥10^6CFU/ml, high sugar consumption)
Diet frequency	Questionnaire results: food consumption amount	0: Maximum of 3 meals per day 1: Maximum of 5 meals per day 2: 7 meals maximum per day 3: More than 7 meals every day
Amount of plaque	Silness-Löe plaque index	0 = Extremely good oral hygiene, PI < 0.4 1 = Good oral hygiene, PI = 0.4-1.0 2 = Less than good oral hygiene, PI = 1.1- 2.0 3= very poor oral hygiene PI >2.0
Streptococcus mutans	Strip mutans test by qPCR	0=Very low (≤20,000 CFU/ml Saliva) 1=Low (20,000-1,00,000 CFU/ml Saliva) 2=Moderate (>1,00,000-1 million CFU/ml Saliva) 3= High (>1 million CFU/ml Saliva)
Fluoride program	Exposure to fluoride	0: Maximum fluoride exposure 1: Fluoride supplements other than toothpaste, however, are used seldom 2. Only fluoride toothpaste 3. No fluoride exposure
Saliva secretion rate	Secretion rate during a provoked saliva test; the examiner's own subjective and clinical evaluation of the patient.	0: Normal saliva secretion 1: Low,0.9–1.1 mL/min 2: Low,0.5–0.9 mL/min 3: Very low, <0.5 mL/min
Saliva buffering capacity	Universal pH indicator	0: Adequate, saliva pH > 6.0 1: Reduced, saliva pH 4.5–5.5 2: Low, saliva pH < 4.0
Clinical judgement	Dental examiner's opinion; the examiner's own clinical and subjective evaluation of the patient.	0: More favorable 1: Usual situation 2: Worse 3: Very high risk of dental caries

Table 4. Sociodemographic characteristics.

	Pregnant	Non-pregnant	Total	P
	No. (%)	No. (%)	No. (%)	
Age (years)				
20-24	42 (42.0)	50 (50.0)	92 (46.0)	
25-29	19 (19.0)	26 (26.0)	45 (22.5)	0.131*
30-34	19 (19.0)	10 (10.0)	29 (14.5)	
35-40	20 (20.0)	14 (14.0)	34 (17.0)	
Mean	27.84 (6.32)	26.54 (5.99)		0.137†
Woman's Education				
Illiterate	8 (8.0)	2 (2.0)	10 (5.0)	
Read & write, and primary	20 (20.0)	15 (15.0)	35 (17.5)	
Intermediate	19 (19.0)	10 (10.0)	29 (14.5)	0.003*
Secondary	28 (28.0)	23 (23.0)	51 (25.5)	
College and above	25 (25.0)	50 (50.0)	75 (37.5)	
Woman's Occupation				
Housewife	83 (83.0)	51 (51.0)	134 (67.0)	
Unskilled manual Worker	5 (5.0)	10 (10.0)	15 (7.5)	
Skilled manual Worker	2 (2.0)	12 (12.0)	14 (7.0)	<0.001*
Non-manual Worker	6 (6.0)	10 (10.0)	16 (8.0)	
High level occupations	4 (4.0)	17 (17.0)	21 (10.5)	
Husband's or Guardian Education				
Illiterate	16 (16.0)	15 (15.0)	31 (15.5)	
Read & write, and primary	15 (15.0)	24 (24.0)	39 (19.5)	
Intermediate	13 (13.0)	15 (15.0)	28 (14.0)	0.462*
Secondary	27 (27.0)	25 (25.0)	52 (26.0)	
College and above	29 (29.0)	21 (21.0)	50 (25.0)	
Husband's or Guardian occupation				
Unemployed	1 (1.0)	2 (2.0)	3 (1.5)	
Unskilled manual Worker	46 (46.0)	43 (43.0)	89 (44.5)	
Skilled manual Worker	24 (24.0)	25 (25.0)	49 (24.5)	0.962*
Non-manual worker	21 (21.0)	23 (23.0)	44 (22.0)	
High rank occupations	8 (8.0)	7 (7.0)	15 (7.5)	
House ownership				
Rented and others	28 (28.0)	8 (8.0)	36 (18.0)	
Partially owned	29 (29.0)	9 (9.0)	38 (19.0)	<0.001*
Owned	43 (43.0)	83 (83.0)	126 (63.0)	
Car ownership				
No	28 (28.0)	28 (28.0)	56 (28.0)	1.000*
Yes	72 (72.0)	72 (72.0)	144 (72.0)	
Income				
Not Sufficient or Marginally Sufficient	12 (12.0)	9 (9.0)	21 (10.5)	
Sufficient	78 (78.0)	78 (78.0)	156 (78.0)	0.664*
Exceeds Daily Needs	10 (10.0)	13 (13.0)	23 (11.5)	
Total	100 (100.0)	100 (100.0)	200 (100.0)	

*By Chi-square test. †By unpaired t-test.

Table 5. Comparison of Cariogram sectors assessed by the Cariogram model among the two study groups.

Cariogram sectors	Pregnant			Non-pregnant			P*
	Mean	(35)	Mean Rank	Mean	(35)	Mean Rank	
Chance to avoid new caries in near future %	50.57	(24.49)	88.51	60.26	(26.39)	112.49	0.003
Circumstances	3.66	(1.88)	110.07	3.12	(1.72)	90.94	0.015
Bacteria	9.02	(4.91)	116.02	6.56	(3.70)	84.98	< 0.001
Susceptibility	31.50	(22.15)	109.49	26.16	(22.55)	91.51	0.028
Diet	5.29	(5.79)	105.25	3.73	(3.59)	95.75	0.235

*By Mann-Whitney test.

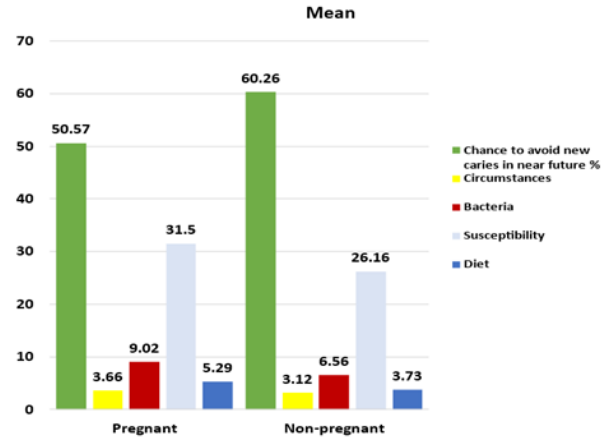

Figure 1. Comparison of Cariogram sectors assessed by Cariogram model among the two study groups.

gnant women (p = 0.011). (Table.6)

The saliva samples showed that the very high and high bacterial colony numbers in the pregnant mouths were higher than in non-pregnant mouths. In 82 pregnant women, there were < 2 x 104 Streptococcus mutans per ml, in 96 non-pregnant women there were < 2 x 104 Streptococcus mutans per ml, indicating very low or zero amounts of mutans streptococci in their saliva, and colonizing about 5% of the tooth surface. In eight pregnant women, there were 2 x 104 -105 Streptococcus mutans per ml, and in one pregnant woman, there were 2 x 104 -105 Streptococcus mutans per ml, indicating low levels of mutans streptococci in saliva and colonizing about 20% of the tooth surface. In five pregnant women, there were > 106 Streptococcus mutans per ml, and in one pregnant woman, there were > 106 Streptococcus mutans per ml, indicating very high amounts of mutans streptococci in the saliva and colonizing more than 80% of the tooth surface.

Based on the saliva sample results, in 77 pregnant women, there were $\leq 10^3$ Lactobacillus in one ml and 96 non-pregnant women there were $\leq 10^3$ Lactobacillus spp. in one ml. In ten pregnant women in one ml, there were $\geq 10^6$ Lactobacillus, and in one non-pregnant woman in one ml, there were $\geq 10^6$ Lactobacillus spp. (Table 7).

Pregnant women had a significantly higher chance of developing caries than non-pregnant women (p < 0.001).

Table 6. Comparison of caries-related factors (Past caries experience, Streptococcus mutans and Lactobacillus spp) among the two study groups.

Caries related factors	Pregnant No. (%)	Non-pregnant No. (%)	Total No. (%)	P value
Past caries experience				
Caries free and no fillings	8 (8.0)	6 (6.0)	14 (7.0)	
Better than normal	**73 (73.0)**	**73 (73.0)**	146 (73.0)	0.952**
Normal for age group	16 (16.0)	18 (18.0)	34 (17.0)	
Worse than normal	3 (3.0)	3 (3.0)	6 (3.0)	
Lactobacillus spp				
Very low (Lactobacillus class 0)	77 (77.0)	96 (96.0)	173 (86.5)	
Low (Lactobacillus class 1)	6 (6.0)	1 (1.0)	7 (3.5)	0.001**
Moderate (Lactobacillus class 2)	7 (7.0)	2 (2.0)	9 (4.5)	
High (Lactobacillus class 3)	10 (10.0)	1 (1.0)	11 (5.5)	
Streptococcus mutans				
Very low (Strip mutans class 0)	82 (82.0)	96 (96.0)	178 (89.0)	
Low (Strip mutans class 1)	8 (8.0)	1 (1.0)	9 (4.5)	0.011**
High (Strip mutans class 2)	5 (5.0)	2 (2.0)	7 (3.5)	
Very high (Strip mutans class 3)	5 (5.0)	1 (1.0)	6 (3.0)	

Table 7. Relationship between the detection level using the qPCR method and the detection of bacteria.

Species	Colony-forming units/ml saliva	Pregnant	Non-Pregnant	Total (number)	Interpretation
Streptococus mutans	$< 2 \times 10^4$	82	96	178	Very little or no mutans streptococci in saliva. Only about 5% of the tooth surface is colonized by bacteria.
	2×10^4 -10^5	8	1	9	Low levels of Mutans streptococci in saliva. About 20% of the tooth surfaces colonized by the bacteria.
	10^5 -10^6	5	2	7	High amount of Mutans streptococci in *saliva*. About 60% of the tooth surfaces are colonized by bacteria.
	$> 10^6$	5	1	6	Very high amounts of Mutans streptococci in the saliva. More than 80% of the tooth surfaces are colonized by bacteria.
Lactobacillus	$\leq 10^3$	77	96	173	Very low sugar consumption
	10^4 -10^5	6	1	7	Low sugar consumption
	10^5-10^6	7	2	9	Moderate sugar consumption
	$\geq 10^6$	10	1	11	High sugar consumption

For instance, 11% of pregnant women had very high risk, 33% had high risk, and 13% had medium risk, compared to 6%, 27%, and 11%, respectively, among non-pregnant women (Figure 2).

Table 8 shows total bacteria presence had a weak positive correlation with caries risk categories. The correlation coefficients for lactobacillus bacteria were 0.189 (pregnant) and 0.152 (non-pregnant) but became significant (0.213) when pregnancy status was not considered. Streptococcus mutans bacteria had a significant positive correlation with caries risk in all three groups: 0.276 (pregnant), 0.284 (non-pregnant), and 0.295 (total cases).

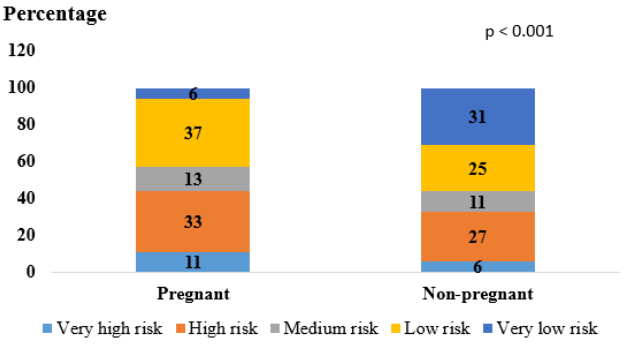

Figure 2. Caries risk categories among the two study groups assessed by Cariogram. *Test By Chi-square test.

Discussion

Maintaining oral hygiene is essential for overall health; untreated tooth decay can lead to various diseases (36). Moreover, pregnancy can affect oral and dental health and increase the risk of tooth decay and loss due to metabolic and hormonal changes (12,37).

The study investigated caries risk in pregnant women using the Cariogram and qPCR methods to determine the amount of Streptococcus mutans and Lactobacillus bacteria in their saliva. The results showed that pregnant women had a significantly higher risk of developing caries than non-pregnant women. The dominant risk factors were susceptibility and bacteria. There was a weak positive correlation between bacteria in saliva and caries risk.

The study found that pregnant women have a higher risk of caries and a lower chance of preventing caries in the near future compared to non-pregnant women, which is consistent with the results of studies by Bardisi et al. (2018), and Elkwatehy (2018). The study emphasizes the importance of using a Cariogram to assess caries risk and supports its effectiveness in predicting future caries in pregnant women (38,39). The results align with previous research published in the literature (31,40–43).

The results of the present study on caries-related factors in pregnant and non-pregnant women were compared with several other studies. Rivera et al. (2019), and Dolić et al. (2017) found that diet and bacteria were the most significant factors in causing caries in pregnant women, followed by susceptibility and circumstances (25,31). Bardisi et al. (2018), showed a non-significant difference in the diet sector between groups, which was consistent with the present study, although the frequency of diet was significant (38). The differences in racial and ethnic origin, diet type and frequency, lifestyle, and sample size could account for the variation in results.

The present study found no difference in past dental caries experiences between pregnant and non-pregnant women, unlike a study in Iraq by Mutlak and Yas (2017), where pregnant women had higher caries parameters. The difference in results may be due to method variations and sample size (44).

The meta-analysis study by Yousefi et al. (2020) included 29 studies and showed that the number of Streptococcus mutans bacteria changes during pregnancy and positively affects caries in pregnant women (45). However, in the present study, it was shown that the very high and high bacterial colony numbers in the pregnant mouth are more than non–the pregnant mouth, but the number of bacterial colonies in most women is very low. This was because, in the present study, the past caries experiences of most women were better than usual, according to the Cariogram model. Therefore, individual variations of the host microflora may be responsible for the differences in S. mutans colonization between studies. Behluli et al. (2021) and Kamate et al. (2017) found that the number of bacterial colonies in the mouth increases as pregnancy age increases and should be considered in future studies (46,47).

Yousefi et al. (2020) conducted a systematic review and meta-analysis to investigate caries risk in pregnant women. They found that pregnant women have a very high risk of caries, which is consistent with the results of the present study. Hormonal changes during pregnancy may contribute to this finding (45).

Rivera et al. (2019) conducted a study in Monte Morelos, Mexico, to investigate how changes in saliva and pH during pregnancy can affect oral health. They examined 53 pregnant and 32 non-pregnant women and found that pregnant women had a higher risk of tooth decay than non-pregnant women. This finding supports the results of the present study (31).

This study found a weak but significant correlation between caries risk categories and the presence of Streptococcus Mutans and Lactobacillus in saliva. As the amounts of these bacteria increased, so did the risk of caries. Other studies have also shown a link between these bacteria and the development of caries (1,48,49).

The results obtained show that Streptococcus mutans (SM) and Lactobacillus (LB) could be detected quantita-

Table 8. The correlation between caries risk groups and the two bacterial levels in saliva.

Bacteria	Caries Risk Categories		
	Pregnant	Non-Pregnant	Total
	Rho (P-Value)	Rho (P-Value)	Rho (P-Value)
lactobacillus_levels	0.189 (0.059)	0.152 (0.131)	0.213 (0.002)
Streptococcus_Mutans	0.276 (0.005)	0.284 (0.004)	0.295 (0.000)

Test By Spearman's rho correlation coefficient.

tively and sensitively by qPCR. Furthermore, SM and LB counts showed a significant positive correlation with caries risk categories, which likely reinforces their relationship with dental caries development. Also, it was shown that these two bacteria can be presented as the most significant microbial agents and initiators of caries in pregnant women.

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Interest conflict
This article assumes that the research and its findings are neutral and that there are no conflicts of interest.

Ethical considerate
The study obtained ethical approval from the Scientific Research Ethical Committee in the College of Dentistry/ Hawler Medical University and followed ethical research considerations. Participants provided written consent after receiving information about the study's objective and assurance that their participation was voluntary and confidential. The study did not use any harmful methodology, and participants had the right to withdraw at any time. The research complied with ethical principles, and the questionnaires were anonymous.

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