Comparative study of Streptococcus mutans in pregnant women's saliva in the first and third trimesters

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Abstract

For the objectives of this study 66 primiparous women were included. Clinical evaluation included the oral hygiene index (OHI), the plaque index (PI), the gingival index (GI), and interdental gingival bleeding index (PBI) in the first and third trimesters. The prevalence of Streptococcus mutans in saliva was determined by a CRT-bacteria diagnostic test.

The OHI value in the third trimester of pregnancy was significantly higher than the value in the first trimester; the GI and the PBI values in third trimester were significantly higher than the first trimester value. The relationship between the OHI index and the prevalence of S. mutans in pregnant women in the first and third trimesters was found to have a weak positive correlation ($p>0.05$). The relationship between PBI and the value of S. mutans in pregnant women in the first and second trimesters showed a moderately weak negative correlation ($p>0.05$).

The results from this study showed differences in the examined parameters, which are important for the early detection and timely treatment of S. mutans infection.

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Introduction

Pregnancy is a physiological condition during which important physical, hormonal, physiological, emotional, and metabolic changes occur. Pregnancy causes various changes in the oral cavity[1], the most common of which are gingival hyperplasia, gingivitis, pyogenic granulomas, dental caries and erosions, and qualitative and quantitative changes in saliva[2].

The role of high circulating estrogen levels is well established and is associated with a higher prevalence of gingivitis and gingival hyperplasia[3].The most common problem during pregnancy is gingivitis. It is estimated that 30% to 75% of pregnant women experience gingivitis to a greater or lesser extent during pregnancy.

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There are numerous reasons for this. Unfortunately, many women who had appropriate oral hygiene before pregnancy, may neglect this part of their self-care, usually unconsciously, during pregnancy. Reasons for this may include nausea, vomiting, weakness, chronic fatigue, and insomnia. These conditions may divert attention from usual hygiene habits. However, when combined with the hormonal changes that accompany pregnancy, dramatic changes in the oral cavity may occur[4,5]. Saliva analysis has become an important resource in determining the physiological and pathological roles of saliva and is a useful tool for disease diagnostics, offering insights based on its origin, composition, function, and interaction with other organic systems.Although harmful processes that accompany periodontal disease (such as bone destruction and periodontal ligament destruction) are associated with bacterial plaque, in general they occur as a result of the host's response to microbial invasion[6]. Elevated levels of *Streptococcus mutans* in the saliva of women during late pregnancy were detected by Pirie *et al*.[7]. The increased prevalence of this bacterium may be the reason for the higher incidence of dental diseases during the late stage of pregnancy.

Streptococcus mutans is considered to be the most prominent dental caries bacteria due to their ability to form biofilms known as plaque on the tooth surfaces[8].

Dietary changes in early pregnancy, such as the regular consumption of sugar between meals and sugary beverages to satisfy cravings or prevent nausea, can cause a drop in the pH of saliva and changes to the teeth and soft tissues in the oral cavity. Numerous studies have described dental and oral changes in pregnant women compared with non-pregnant women[9,10].Periodontal diseases are closely connected to the general health of an individual[11,12]. Dental plaque microorganisms (which include more than 700 different types of microorganisms) play a vital role in the development and progression of periodontitis[11].
The aim of our current research was to assess the impact of *S. mutans* in saliva on the oral hygiene and degree of gingival inflammation in pregnant women in the first and third trimesters of pregnancy.

Materials and methods

Subjects

This study included a sample of 66 pregnant women who were undergoing their first pregnancy and lived in or around Pristina (Kosovo). All subjects were treated in the Department of Gynecology and Obstetrics at the University Clinical Center, Prishtina, Kosovo. Women classified as undergoing a high-risk pregnancy and pregnant women with soft tissue changes in their oral cavity were excluded from the study.

Design of the study

Clinical examinations

Clinical examinations consisted of the following clinical procedures: recording a detailed clinical history of the patient and determining their condition and gestational age (weeks/months of pregnancy), determining their oral hygiene, and noting the basic condition of the periodont.

Clinical history of the patient

A detailed clinical history was obtained for each patient according to previously established protocols for guiding pregnancy.

Indexes to measure oral health

The soft deposits index of the teeth (the oral hygiene index, OHI) was determined according to theGreen–Vermillion scoring system[11], as follows:

0 - no soft deposits (plaque);

1 - soft deposits (plaque) localized only in the gingival third of the tooth crown;

2 - soft deposits (plaque) cover more than one-third but less than two-thirds of the tooth crown surface;

3 - very poor oral hygiene (soft deposits covering more than two-thirds of the crown surface).

The gingival index (GI) was determinedaccording to Silness-Löe[13] based on the following scoring system:

0 - normal gingiva;

1 - mild inflammation;

2 - moderate inflammation;

3 - severe inflammation.

The index of interdental gingival bleeding (papila bleeding index, PBI)was determinedaccording to the guidelines of Ainamo[14] based on the following scoring system:

0 - no evidence of hemorrhages on examination;

1 - hemorrhage evident only on one place after probing;

2 - linear or multiple points of bleeding from the papilla;

3 - interdental space filled with blood immediately after probing;

4 - excessive bleeding after probing.

Collection of saliva samples

Saliva was collected in sterile tubes, early in the morning, between 8 and 10 a.m., at least two hours after a meal, after teeth brushing, and without the use of rinse aid.

Assessment of Streptococcus mutans *in saliva*

The abundance of *S. mutans* in saliva was determined by a diagnostic test, CRT-bacteria (Vivadent, Schaan, Lihtenstein), strictly following the manufacturer's instructions. We also used the Dentocult SM system. This method is based on the use of a selected liquid culture and the application of *S. mutans* onto a test strip. The SM test strip discriminates the ability to develop on a solid surface in combination with a selected liquid culture (high sucrose concentration with bacitracin).Dentocult LB media included a paraffin tablet to stimulate saliva. Bacitracin, which prevents bacterial growth, was added to the saliva at least 15 minutes before use. Strips were divided into four test groups and a control in sterile tubes containing selective LB media, and the number of *S. mutans* colonies / ml saliva was determined.

The density of SM colonies (CFU / ml) in saliva was determined according to the following scoring system: 0, <10^3 CFU / ml saliva, only about 5% of the crown surface is colonized by bacteria; 1, 10^4 CFU / ml saliva (low SM level), about 20% of the surface of the crown is colonized with bacteria; 2, 10^5 CFU / ml in saliva (high presence of CMU in saliva), about 60% of the surface of the crown is colonized by bacteria; 3, 10^6 CFU / ml in saliva (very large amount of MS in saliva), about 80% of the surface of the crown is colonized by bacteria.All microbiological analyses were performed in the Department of Microbiology and Parasitology at the University Clinical Center, Prishtina, Kosovo.

Statistical analysis

Statistical analysis of the data was performed using a variety of methods, as detailed below, and the software Statistica 7.1 for Windows and SPSS Statistics 23.0. For the analysis of series with characteristics attributable to *S. mutans*, the percentages of the structure that were affected were determined (%). Differences in series with characteristics attributable to the first and third trimesters were tested using Fisher's Exact test / Monte Carlo significance test (two-sided) (p values). Differences between the first and third trimesters were analyzed using the Wilcoxon matched pairs test (Z / p). A p value <0.05 was considered to indicate statistical significance. The data are presented in tabular and graphical form.

Results

The values of OHI, GI, and PBI were determined for pregnant women in their first trimester of pregnancy. The OHI ranged from 1.02 ± 0.54; ± 95.00% CI: 0.88–1,15; the GI value ranged from 0.33 ± 0.64; 95.00% CI: 0.18–0.49; and the PBI was 0.61 ± 0.94; ± 95.00% CI: 0.37–0.84.

For pregnant women in their third trimester of pregnancy, the OHI ranged from 1.65 ± 0.77; 95.00% CI: 1.46–1.84; the GI value was 0.64 ± 0.72; ± 95.00% CI: 0.46–0.81; and the PBI ranged from 1.18 ± 0.96; ± 95.00% CI: 01.42 (Table 1).

First trimester	Valid N	Mean	Confidence -95,00%	Confidence +95,00	Minimum	Maximum	Std.Dev.
OHI	66	1,02	0,88	1,15	0	3	0,54
GI	66	0,33	0,18	0,49	0	3	0,64
PBI	66	0,61	0,37	0,84	0	3	0,94
Third trimester							
OHI	66	1,65	1,46	1,84	1	3	0,77
GI	66	0,64	0,46	0,81	0	2	0,72
PBI	66	1,18	0,95	1,42	0	2	0,96

Table 1. Values of OHI, GI and PBI at the first and third trimester.

First trimester	Valid N	Mean	Confidence -95,00%	Confidence +95,00	Minimum	Maximum	Std.Dev.
OHI	66	1,02	0,88	1,15	0	3	0,54
GI	66	0,33	0,18	0,49	0	3	0,64
PBI	66	0,61	0,37	0,84	0	3	0,94
Third trimester							
OHI	66	1,65	1,46	1,84	1	3	0,77
GI	66	0,64	0,46	0,81	0	2	0,72
PBI	66	1,18	0,95	1,42	0	2	0,96

Table 2. Comparison of OHI, GI,PBI at the first trimester and third trimester.

The differences in the values of the OHI, GI, and PBI in pregnant women in their first trimester compared with the third trimester of pregnancy were as follows. The OHI value in the third trimester of pregnancy was significantly higher than in the first trimester [Z=4.46 and p<0.001 (p=0.000)]; GI in the third trimester of pregnancy was significantly higher than in the first trimester [Z=2.72 and p<0.01 (p=0.006)]; and PBI in the third trimester of pregnancy was significantly higher than in the first trimester (Z=3.46 and p<0.001) (Table 2).

The data relating to the detection of *S. mutans* in the saliva of pregnant women in their first trimester of pregnancy indicated that out of a total of 66 pregnant women, one (1.50%) woman had negligible levels (<10^3 CFU / ml saliva), 14 (21.20%) had low levels (10^4 CFU / ml saliva), 39 (59.10%) had high levels (10^5 CFU / ml saliva), 10 (15.20%) had very high levels (10^6 CFU / ml saliva) of *S. mutans* in saliva, and in two (3.00%) women, 80.00% of the crown surface was colonized with bacteria (Table 3).

First trimester		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Negligible values	1	1,5	1,5	1,5
	Low level of SM	14	21,2	21,2	22,7
	Large presence of SM	39	59,1	59,1	81,8
	Very large amounts of SM	10	15,2	15,2	97,0
	High colonization with SM	2	3,0	3,0	100,0
	Total	66	100,0	100,0	

Table 3. Streptococcus mutans values at the first trimester.

The data presented refer to the detection of *S. mutans* in saliva in pregnant women in the third trimester of pregnancy. Of the 66 pregnant women studied, 7 (10.60%) had a low level (10^4 CFU / ml saliva) of *S. mutans* in their saliva, 39 (59.10%) had a high level (10^5 CFU / ml saliva), and 20 (30.30%) had a very high level (10^6 CFU / ml saliva) of *S. mutans* in their saliva (Table 4).

Third trimester		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Low level of SM	7	10,6	10,6	10,6
	Large presence of SM	39	59,1	59,1	69,7
	Very large amounts of SM	20	30,3	30,3	100,0
	Total	66	100,0	100,0	

Table 4. Streptococcus mutans values at the third trimester.

The cross-tabulation of S. mutans values obtained for the first and third trimesters of pregnancy revealed that one (100.00%) woman who showed negligible values of *S. mutans* in her saliva in the first trimester (<10^3 CFU / ml saliva), showed high levels in the third trimester (10^5 CFU / ml saliva). Of the 14 (100.00%) women who showed low levels of *S. mutans* in saliva in the first trimester (10^4 CFU / ml saliva), one (7.10%) woman showed low levels (10^4 CFU / ml saliva), 7 (50.00%) women showed high levels (10^5 CFU / ml saliva), and 6 (42.90%) women showed very large levels (10^6 CFU / ml saliva) of *S. mutans* in saliva in the third trimester. Of the 39 (100.00%) women who showed high levels of *S. mutans* in the first trimester (10^5 CFU / ml saliva), 5 (12.80%) women showed low levels (10^4 CFU / ml saliva), 23 (59.00%) women showed high levels (10^5 CFU / ml saliva), and 11 (28.20%) women showed very high levels (10^6 CFU / ml saliva) of *S. mutans* in their saliva in the third trimester.

Of the 10 (100.00%) pregnant women who showed very high levels (10^6 CFU / ml saliva) of *S. mutans* in their saliva in the first trimester, 7 (70.00%) women showed high levels (10^5 CFU / ml saliva) and 3 (30.00%) women showed very high levels (10^6 CFU / ml saliva) of *S. mutans* in their saliva in the third trimester.

Of the two (100.00%) pregnant women for whom 80.00% of the crown surface was colonized with bacteria in the first trimester, one (50.00%) showed low levels (10^4 CFU / ml saliva) and one (50.00%) showed high levels (10^5 CFU / ml saliva) of *S. mutans* in their saliva in the third trimester.

The cross-tabulation of *S. mutans* values for the first and third trimesters of pregnancy revealed a Fisher's exact test value of 6,409 and a p value >0.05 (p=0.645), and a Monte Carlo significance test (two-sided) value of 0.632–0.657, indicating no significant difference (Figure 1).

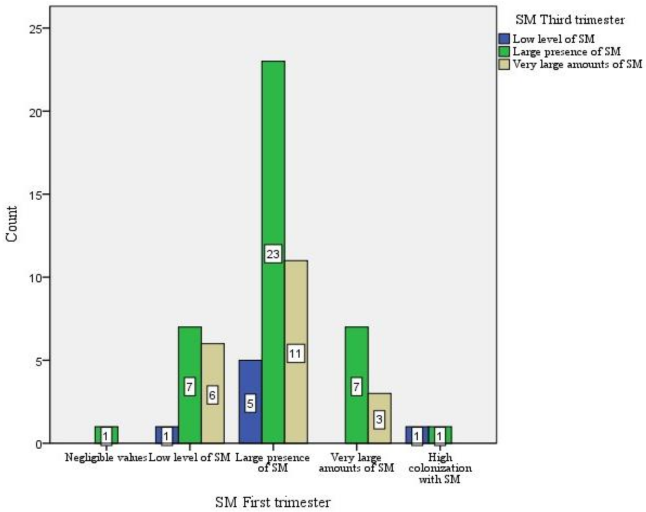

Figure 1. Comparison of Streptococcus mutans at the first trimester and the third trimester.

Correlations

OHI index * *S. mutans* / First trimester

The relationship between the OHI index and the prevalence of *S. mutans* in pregnant women in the first trimester showed a weakly positive correlation that was not statistically significant (Spearman's rank order R=0.05 and p>0.05). With increased levels of *S. mutans* in saliva in the first trimester, the OHI index value increased but not statistically significantly.

OHI index * *S. mutans* / Third trimester

The relationship between the OHI index and the prevalence of *S. mutans* in pregnant women in the third trimester showed a weakly positive correlation that was not statistically significant (Spearman's rank order R=0.05 and p>0.05). With increased levels of *S. mutans* in saliva in the third trimester, the OHI index values increased but not significantly.

GI * *S. mutans* / First trimester

The relationship between GI and the prevalence of *S. mutans* in pregnant women in the first trimester showed a weakly positive correlation that was not statistically significant (Spearman's rank order R=0.20 and p>0.05). With increased levels of *S. mutans* in saliva in the first trimester, GI values increased but not significantly.

GI * *S. mutans* / Third trimester

The relationship between GI and the prevalence of *S. mutans* in pregnant women in the third trimester showed a very weak negative correlation that was not statistically significant (Spearman's rank order R=-0.01 and p>0.05). With increased levels of *S. mutans* in saliva in the third trimester, GI values decreased but not significantly.

PBI * *S. mutans* / First trimester

The relationship between PBI and the prevalence of *S. mutans* in pregnant women in the first trimester showed a moderately weak negative correlation that was not statistically significant (Spearman's rank order R=-0.20 and p>0.05). With increased levels of *S. mutans* in saliva in the first trimester, PBI values decreased but not significantly.

PBI * *S. mutans* / Third trimester

The relationship between PBI and the prevalence of *S. mutans* in pregnant women in the third trimester showed a weak negative correlation that was not statistically significant (Spearman's rank order R=-0.06 and p>0.05). With increased levels of *S. mutans* in saliva in the third trimester, PBI values decreased but not significantly.

Discussion

Our data indicated differences in the values of OHI, GI, and PBI in pregnant women in the first and third trimesters of pregnancy. This finding of significantly higher values in the third trimester of pregnancy correlated with the findings of previous studies by Vittek *et al*. and Lapp *et al*.which suggested that hormonal activity during pregnancy may predispose pregnant women to gingivitis and periodontitis[15,16]. They pointed to progesterone as the possible cause of local inflammation. Löe*et al*. and Miyazaki *et al*. reported that the frequency of periodontitis increased from the first to the third trimester, again suggesting that the periodontal tissues may be predisposed to periodontitis during pregnancy[17,18].Decreased oral hygiene in our subjects during the third trimester may be due to reduced self-care during the late stages of pregnancy and an unawareness of the particular importance of limiting the accumulation of dental plaque during pregnancy. This finding was in accordance with the findings of Agbelusi *et al*. and Pirie *et al*., who associated gingival changes in early pregnancy with dietary changes, such as the increased consumption of sugary drinks and sweets, often to prevent nausea, that contribute to a lower pH of the saliva[19,7].In a study by Ho *et al*. looking at periodontal tissue changes in pregnant and non-pregnant women, gingival inflammation was found to be statistically significantly higher (p<0.001) in pregnant women in the third trimester[20]. The authors connected gingival inflammation in pregnant women to the increased deposition of dental plaque biofilms. According to Pirie *et al*. the reduced maintenance of oral hygiene increases the abundance of anaerobic bacteria in saliva and in periodontal tissues and can cause worsening of the inflammatory reaction[7]. This is consistent with the results obtained in our study. They also pointed out that along with the increase in the OHI value, there was a progressive increase in the GI index value in all subjects[7]..Van der Reijden *et al*. showed that microorganisms associated with periodontitis can coexist with *S. mutans* and survive under acidic conditions, limited in their mutual interactions[21]. Therefore, individual variations of the host microflora may be responsible for the differences in *S. mutans* colonization between studies.Dental biofilm accumulates on all available solid tooth surfaces, immediately after its removal by mechanical or chemical substances. If non-pathogenic bacterial flora is present and there is no invasion of potentially pathogenic microorganisms, the development of periodontal inflammation will not occur. However, if dental biofilm accumulates, the initial lesion indicating gingivitis develops after 24 hours[11].

Conclusions

The data from our study indicate poorer oral hygiene condition at the third trimesters of pregnancy. To avoid oral disease during the late stages of pregnancy, appropriate prevention measures are required in a timely manner, including maintaining high standards of oral hygiene andnormal eating habits.One way to maintain effective oral and dental hygiene is by gargling using mouthwash. The use of mouthwash is one of the earliest prevention methods for caries due to reduced plaque attachment[22].

Declaration of Interest

The authors report no conflict of interest.

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