Correlations of Lactobacillus in Saliva and OHI, PI, GI and PBI Indices in Pregnant Women

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ABSTRACT

Pregnancy is a completely new physiological condition that stimulates important protective forces of the future mother. The changes that occur in the body of the pregnant woman are hormonal, biochemical, anatomical, and histological which are manifested in the functions of all organs. This study included 66 pregnant women in their first pregnancy living in Pristina or coming from nearby places in Kosovo. It aimed at determining the Oral Hygiene Index (OHI), Plaque Index (PI), Gingival index (GI) and Index of interdental gingival bleeding (PBI) in pregnant women in the first and third trimester of pregnancy. The amount of Lactobacillus in saliva was determined by the diagnostic test of CRT-bacteria. The results show that OHI value in the third trimester of pregnancy for (p = 0.000) was significantly higher than the value in the first trimester; IDP value in the third trimester of pregnancy for (p=0.000) was significantly higher than the first trimester value; GI value in the third trimester of pregnancy was significantly higher than in the first trimester (p=0.000). A weak positive correlation was obtained between the OHI index and Lactobacillus in pregnant women in the first and third trimesters (p> 0.05). The correlation between IDP and the value Lactobacillus in pregnant women in the first and third trimesters showed a weak positive value (p> 0.05). The correlation between GI and the value of Lactobacillus in pregnant women in the first and third trimester showed a weak negative insignificant correlation for Spearman Rank Order R = –0.05 and p> 0.05. The results obtained from this study pointed at small differences in the examined parameters which are very important for early detection and timely prevention.

Key words: saliva, pregnancy, Lactobacillus, OHI, PI, GI, PBI index.

Introduction

Periodontal diseases are among the most common oral infectious diseases associated with the establishment of a highly pathogenic biofilm that triggers an immune/inflammatory host response, leading to the destruction of supporting periodontal tissues and eventual tooth loss.¹²

Oral bacteria and periodontal infections have been indicated as potential risk factors for several systemic diseases.¹³ Due to the anatomical proximity of the periodontal biofilm to the gingival bloodstream, periodontal pockets may act as reservoirs of microbial pathogens and their products, as well as inflammatory mediators and immune complexes that may disseminate to other sites of the human body.⁵,⁷ The periodontal microbiota plays a major role in the establishment of periodontal health and the development of periodontal diseases. This microbiota comprises mostly commensal resident members of oral species that have co-evolved to colonize the human oral cavity.⁸–¹⁰

The changes that occur in the body of the pregnant woman are hormonal, biochemical, anatomical, and histological which are manifested in the function of all its organs as well as in the oral cavity.¹¹ Among the most common are gingival hyperplasia, gingivitis, pyogenic granulomas, dental caries and erosions, and qualitative and quantitative changes in saliva.¹² The role of high circulating estrogen levels is well established and is associated with a higher prevalence of gingivitis and gingival hyperplasia.¹³
During pregnancy the most common problem is gingivitis. It is estimated that 30 to 75% of pregnant women have gingivitis to a greater or lesser extent during pregnancy. The reasons for this are multiple. Many women reduced care to maintain oral hygiene during pregnancy, among reasons for this can be nausea, vomiting, weakness, feeling of chronic fatigue, insomnia, etc. These conditions in pregnant women divert attention from the usual hygiene habits for which pregnant women think are not primary or of vital importance. Such an approach followed by hormonal changes that accompany pregnancy can often lead to significant dramatic changes in the oral cavity.¹¹⁵

The saliva analysis has become an important resource for the determination of the role of saliva in physiological and pathological implications and is a useful tool for disease diagnostics mainly due to its origin, composition, function, and interaction with other organic systems.

Although harmful processes that accompany periodontal diseases (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 this microbial invasion.¹⁶ Due to that fact, the aim of our research is based on the latest literature research and own clinical findings to provide data for assessment and impact of Lactobacillus in saliva on the oral hygiene level and the degree of gingival inflammation in pregnant women in the first and third trimester of pregnancy.

**Materials and Methods**

The study included 66 pregnant women in their first pregnancy, as dependent samples, which live in Pristina (Kosovo) or come from the surrounding areas. All subjects are treated in the Department of Gynecology and Obstetrics, University Clinical Center, Pristina, Kosovo. Pregnant women with high-risk pregnancies and pregnant women with soft tissue changes in the oral cavity were excluded from the study.

The research protocol was approved by the Ethical Committee of Medical Faculty, University of Pristina “Hasan Pristina, Ref. nr. 4096, 07. 06. 2019.

**Clinical examinations**

Clinical examinations consisted of the following clinical procedures: obtaining detailed anamnesis of the patient and determining the condition and gestational age (weeks/months of pregnancy), determining oral hygiene, and determining the basic periodontal condition. Detailed patient anamnesis was obtained according to the previously established protocols for guiding pregnancy.

**Examined indices**

Soft Plaque Index of the teeth (OHI- Oral Hygiene Index) was determined according to the Green-Vermillion¹⁷ scoring system and it consisted of the following: Score 0 – no soft deposits (plaque); Score 1 – soft deposits (plaque) are localized only in the gingival third of the tooth crown; Score 2 – soft deposits (plaque) cover more than one third but less than two-thirds of the crown surface; Score 3 – very poor oral hygiene (soft deposits covering more than two-thirds of the crown surface).

Plaque Index (PI) according to Löe-Silness¹⁸ is expressed in numbers and the interpretation was: Score 0 – no deposits (plaque) at the crown surface of the tooth; Score 1 – a thin layer of plaque on the crown of the tooth near the gingiva, detected by probe; Score 2 – moderate amount of plaque on the tooth crown macroscopically visible; Score 3 – a large amount of plaque that filled the interdental space.

Gingival index (GI) according to Löe-Silness was interpreted as: Score 0 – normal gingiva; Score 1 – mild inflammation; Score 2 – moderate inflammation; Score 3 – severe inflammation.

Index of interdental gingival bleeding (Papilla Bleeding Index – PBI) is interpreted following the guidelines of Ainamo²⁶: Score 0 – No evident hemorrhage after examination; Score 1 – Hemorrhage is evident only in one place after probe; Score 2 – There is linear or multiple bleeding points from the papilla; Score 3 – Interdental spaces filled with blood immediately after the probing; Score 4 – Excessive bleeding after probing.

**Estimation of the Lactobacillus amount in saliva**

Saliva was collected in special sterile tubes, early in the morning between eight and ten o’clock a.m., at least two hours after the meal and after teeth brushing without the use of rinse aid.

The amount of Lactobacilli in saliva was determined by a diagnostic test of CRT-bacteria (Vivadent, Schaan, Lichtenstein) where we strictly followed the manufacturer’s instructions. Dentocult LB – includes a paraffin tablet to stimulate saliva, Bacitracin, which prevents the bacterial growth, which we added to the saliva at least 15 minutes before use, a strip containing selective Lactobacillus agar on all sides, a test strip showing lactobacillus colonies/ml in saliva, which was divided into four classes and test sterile tubes. We used an incubator located in the Department of Microbiology and Parasitology at the Clinical Hospital in Pristina.

Lactobacillus test data were interpreted as follows: Score 0: Very low consumption of cariogenic foods and <10⁸ (CFU) / ml colony with a higher lactobacilli number; Score 1: Low consumption of fermented carbohydrates and cariogenic diet 10⁴ CFU / ml; Score 2: Moderate consumption of fermented carbohydrates and cariogenic foods 10⁵ CFU / ml; Score 3: Consumption of highly fermented carbohydrates and inadequate foods> 10⁶ CFU / ml.

**Statistical analysis**

The data analysis was performed with the statistical program Statistica 7.1 for Windows and SPSS Statistics 23.0. The following methods were applied: In the analysis
of the series with attributive characteristics of Lactobacil-
lus, the percentages of the structure were determined (%);
The differences in the series with attributive characteris-
tics in the correlation between the first and third trimes-
ter were tested using the Fisher Exact test / Monte Carlo
Sig. (2-sided), (p); Differences in the correlation between
the first and third trimester tested with Wilcoxon Matched
Pairs Test (Z / p); Significance is determined by p <0.05.
The data are presented in tabular and graphical form.

Results

Descriptive statistics

The results of the study related to the assessment of
Lactobacillus in the saliva of pregnant women in the first
trimester of pregnancy, out of a total of 66 pregnant wom-
en, in 33 (50.00%) registered low consumption of fer-
mented carbohydrates and cariogenic foods (<10^4  CFU / ml / formed colonies with Lactobacillus number), 24
(36.40%) had moderate consumption of fermented carbo-
hydrates and cariogenic foods (10^5 CFU / ml / formed col-
onies with Lactobacillus number) and in 9 (13.60%) of
pregnant women consumption of highly fermented carbo-
hydrates and inadequate nutrition (> 10^6 CFU / ml / formed colonies with Lactobacillus number was reported).

The differences in the values of OHI, IDP, GI, and PBI
in pregnant women in the correlation between the first
and third trimester of pregnancy for OHI value in the
third trimester of pregnancy for Z = 4.46 and p <0.001 (p
= 0.000) are significantly higher than the value in the first
trimester; the value of IDP in the third trimester of preg-
nancy for Z = 4.52 and p <0.001 (p = 0.000) is significant-
ly higher than the value in the first trimester; the value

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in pregnant women in the correlation between the first
and third trimester of pregnancy for OHI value in the
third trimester of pregnancy for Z = 4.46 and p <0.001 (p
= 0.000) are significantly higher than the value in the first
trimester; the value of IDP in the third trimester of preg-
nancy for Z = 4.52 and p <0.001 (p = 0.000) is significant-


TABLE 1

<table>
<thead>
<tr>
<th>Index</th>
<th>Valid N</th>
<th>Mean</th>
<th>Confidence -95,00%</th>
<th>Confidence +95,00%</th>
<th>Min.</th>
<th>Max.</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHI</td>
<td>66</td>
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<td>0.88</td>
<td>1.15</td>
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<td>3</td>
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<td>1.08</td>
<td>0.93</td>
<td>1.22</td>
<td>0</td>
<td>3</td>
<td>0.59</td>
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<td>GI</td>
<td>66</td>
<td>0.33</td>
<td>0.18</td>
<td>0.49</td>
<td>0</td>
<td>3</td>
<td>0.64</td>
</tr>
<tr>
<td>PBI</td>
<td>66</td>
<td>0.61</td>
<td>0.37</td>
<td>0.84</td>
<td>0</td>
<td>3</td>
<td>0.94</td>
</tr>
<tr>
<td>Third trimester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHI</td>
<td>66</td>
<td>1.65</td>
<td>1.46</td>
<td>1.84</td>
<td>1</td>
<td>3</td>
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<tr>
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<td>1.90</td>
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<tr>
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<td>0.46</td>
<td>0.81</td>
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<td>2</td>
<td>0.72</td>
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<tr>
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<td>0.95</td>
<td>1.42</td>
<td>0</td>
<td>2</td>
<td>0.96</td>
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</table>

The results of the study related to the assessment of
Lactobacillus in saliva in pregnant women in the third tri-
mester of pregnancy out of a total of 66 pregnant wom-
en, in 40 (60.60%) low consumption of fermented carbo-
hydrates and cariogenic foods (<10^4 CFU / ml / formed colonies with number of Lactobacillus) was reported).
had moderate consumption of fermented carbohydrates and cariogenic food \((10^5 \text{ CFU} / \text{ml} / \text{formed colonies with number of Lactobacillus})\) and in 6 (9.10%) pregnant women consumption of highly fermented carbohydrates and inadequate nutrition \( (> 10^6 \text{ CFU} / \text{ml} / \text{formed colonies with number of Lactobacillus})\) was reported. (Table 4).

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>LACTOBACILLUS / THIRD TRIMESTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>Percent</td>
</tr>
<tr>
<td>Low consumption of FC</td>
<td>40</td>
</tr>
<tr>
<td>Moderate consumption of FC</td>
<td>20</td>
</tr>
<tr>
<td>High consumption of FC</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
</tr>
</tbody>
</table>

The presented cross-tabulation of Lactobacillus values in the correlation between the first and third trimester of pregnancy is as follows, from 33 (100.00%) women who in the first trimester had <10^4 (CFU / ml / formed colonies with number of Lactobacillus), in the third trimester 19 (57.60%) were with <10^4 (CFU / ml / formed colonies with number of Lactobacillus), 11 (33.30%) pregnant women were with 10^5 CFU / ml / formed colonies with a number of Lactobacillus) and 3 (9.10%) with > 10^6 CFU / ml / formed colonies with a number of Lactobacillus).

Of the 24 (100.00%) women who in the first trimester had moderate consumption of fermented carbohydrates and cariogenic foods \((10^5 \text{ CFU} / \text{ml} / \text{formed colonies with Lactobacillus number})\), in the third trimester 15 (62.50%) pregnant women were with <10^4 (CFU / ml / formed colonies with number of Lactobacillus), 6 (25.00%) pregnant women were with 10^5 CFU / ml / formed colonies with number of Lactobacillus) and 3 (12.50%) with > 10^6 CFU / ml / formed colonies with number of Lactobacillus).

From 9 (100.00%) women who in the first trimester had a consumption of highly fermented carbohydrates and inadequate nutrition \( (> 10^6 \text{ CFU} / \text{ml} / \text{formed colonies with number of Lactobacillus})\), in the third trimester in 6 (66.70%) <10^4 (CFU / ml / formed colonies with number of Lactobacillus were confirmed) and 3 (33.30%) pregnant women had values of 10^5 CFU / ml / formed colonies with number of Lactobacillus). In the presented cross-tabulation of the values of Lactobacillus in the correlation between the first and third trimester of pregnancy for Fisher's Exact Test = 1.331 and p > 0.05 (p = 0.914) / Monte Carlo Sig. (2-sided) / 0.907 – 0.921 / there is no significant difference. (Table 5).

**Correlations**

**OHI index * Lactobacillus / First trimester**

The correlation between OHI index and the value of Lactobacillus in pregnant women in the first trimester, a weak positive insignificant correlation was observed for Spearman Rank Order \( R = 0.12 \) and \( p > 0.05 \). As values rise (consumption of fermented carbohydrates and cariogenic foods) of Lactobacillus in the first trimester the values of the OHI index increased insignificantly. (Figure 1)

**IDP * Lactobacillus / Third trimester**

The ratio between IDP and Lactobacillus value in pregnant women in the third trimester, for Spearman Rank Order \( R = 0.02 \) and \( p > 0.05 \), a very weak positive insignificant correlation was observed, with rising values (consumption of fermented carbohydrates and cariogenic food) of Lactobacillus in the third trimester insignificantly the values of the OHI index increased insignificantly. (Figure 2)

<table>
<thead>
<tr>
<th>TABLE 5</th>
<th>LACTOBACILLUS / FIRST TRIMESTER / THIRD TRIMESTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB Third trimester</td>
<td>Low consumption of FC</td>
</tr>
<tr>
<td>Count</td>
<td>19</td>
</tr>
<tr>
<td>%</td>
<td>57.6%</td>
</tr>
<tr>
<td>LB First trimester</td>
<td>Moderate consumption of FC</td>
</tr>
<tr>
<td>%</td>
<td>62.5%</td>
</tr>
<tr>
<td>High consumption of FC</td>
<td>Count</td>
</tr>
<tr>
<td>%</td>
<td>66.7%</td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
</tr>
<tr>
<td>%</td>
<td>60.6%</td>
</tr>
</tbody>
</table>
of the values (consumption of fermented carbohydrates and cariogenic foods) of Lactobacillus in the first trimester. IDP values increased insignificantly (Figure 3)

**IDP * Lactobacillus / Third trimester**

The ratio between IDP and the value of Lactobacillus in pregnant women in the third trimester, very weak positive insignificant correlation was observed for Spearman Rank Order $R = 0.04$ and $p> 0.05$, as with rising values (consumption of fermented carbohydrates and cariogenic foods) of Lactobacillus in the third trimester the values of IDP increased insignificantly (Figure 4).

**GI * Lactobacillus**

Spearman Rank Order $R = 0.02(p>0.05)$

**OHI index * Lactobacillus**

Spearman Rank Order $R = 0.12(p>0.05)$

**PBI * Lactobacillus**

Spearman Rank Order $R = –0.32$ and $p < 0.05$ a moderately strong negative significant correlation was found, with increasing of the values (consumption of fermented carbohydrates and cariogenic food) of Lactobacillus in the third trimester there was a significant decrease of PBI values (Figure 8).
Discussion

In this study, we assessed the prevalence and counts of pathogens in the subgingival area in pregnant women in the first and third trimesters of pregnancy.

The values obtained for the examined parameters from our study indicated that the differences in the values of OHI, IDP, GI, and PBI in pregnant women in the correlation between the first and third trimester of pregnancy for the value of OHI in the third trimester of pregnancy for p <0.001 (p = 0.000) is significantly higher than the value in the first trimester; the value of IDP in the third trimester of pregnancy by p <0.001 (p = 0.000) is significantly higher than the value in the first trimester; the value of GI in the third trimester of pregnancy for p <0.01 (p = 0.006) is significantly higher than the value in the first trimester; the value of PBI in the third trimester of pregnancy for p <0.001 (p = 0.000) is significantly higher than the value in the first trimester.

The results of our study were of significantly higher values in the third trimester of pregnancy which correlated with the studies of Vittek et al.,20 and Lapp et al.21 that link this condition to the hormonal activity during pregnancy that may predispose pregnant women to gingivitis and periodontitis. They point out progesterone as the possible cause of local inflammation. Loe et al.22 and Miyazaki et al.23 in their study regarding the changes in these parameters between pregnant and non-pregnant women observed that the frequency of periodontitis increased from the first to the third trimester. As a possible reason, they indicated the predisposition of the periodontal tissues during pregnancy.

Decreased oral hygiene in our subjects in the third trimester is due to reduced care and ignorance of the oral health issues that lead to the accumulation of dental plaque during pregnancy. This finding was in accordance with the findings of Agbelusi et al.24 and Pirie et al.25, who associated gingival changes in early pregnancy with dietary changes, such as sugary drinks and sweets, mainly taken to prevent nausea but also contribute to lower salivary pH.

In studies by Ho et al.26 for periodontal tissue changed 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. The
The correlation between IDP and the value of Lactobacillus in pregnant women in the first and third trimesters for \( p > 0.05 \) found a weak positive correlation. Of Lactobacillus in pregnant women in the first and third trimester. However, timely pathogenic microorganisms, the development of periodontal inflammation will not occur. However, if dental biofilm accumulates, the initial lesion of gingivitis develops after 24 hours.28

**Conclusion**

The obtained results of the oral hygiene examination and the gingival indices indicate small differences in the examined parameters between the first and third trimester of pregnancy, except that there were differences for Lactobacillus between the examined trimesters. Significant cross-tabulation was found in the ratio between PBI and Lactobacillus in the third trimester. However, timely and appropriate prevention, proper oral hygiene, and change of eating habits are recommended.

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**REFERENCES**


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KORELACIJA BAKTERIJA LACTOBACILLUS U SLINI I OHI, PI, GI I PBI INDEKSA U TRUDNICA

SAŽETAK

Trudnoća je potpuno novo fiziološko stanje koje potiče važne zaštitne snage buduće majke. Promjene koje se događaju u tijelu trudnice su hormonalne, biokemijske, anatomske i histološke koje se očituju u funkcijama svih organa. Ovo istraživanje obuhvatilo je 66 trudnica u prvoj trudnoći koje žive u Prištini ili dolaze iz obližnjih mjesta na Kosovu. Cilj mu je bio determinirati indeks oralne higijene (OHI), indeks plaka (PI), gingivalni indeks (GI) i indeks interdentalnog gingivalnog krvarenja (PBI) u trudnica u prvom i trećem tromjesečju trudnoće. Količina Lactobacillusa u slini određena je dijagnostičkim testom CRT za određivanje razine bakterija. Rezultati pokazuju da je vrijednost OHI u trećem tromjesečju trudnoće za (p = 0,000) bila značajno viša od vrijednosti u prvom tromjesečju; Vrijednost IDP-a u trećem tromjesečju trudnoće za (p=0,000) bila je značajno viša od vrijednosti u prvom tromjesečju; Vrijednost GI u trećem tromjesečju trudnoće bila je značajno viša nego u prvom tromjesečju p<0,01 (p=0,006); Vrijednost PBI u trećem tromjesečju trudnoće bila je značajno viša nego u prvom tromjesečju (p=0,000). Dobivena je slaba pozitivna korelacija između OHI indeksa i Lactobacillusa u trudnica u prvom i trećem tromjesečju (p>0,05). Korelacija između GI i vrijednosti Lactobacillusa u trudnica u prvom i trećem tromjesečju pokazala je slabu pozitivnu vrijednost (p>0,05). Korelacija između GI i vrijednosti Lactobacillusa u trudnica u prvom i trećem tromjesečju pokazala je slabu negativnu beznačajnu korelaciju za Spearman Rank Order R = –0,05 i p>0,05. Rezultati dobiveni ovim istraživanjem ukazali su na male razlike u ispitivanim parametrima koji su vrlo važni za rano otkrivanje rizika od karijesa i pravovremenu prevenciju.