BACKGROUND & OBJECTIVE: Sialometric and sialochemical analyses during pregnancy are not consistent, and frequently contradictory in terms of salivary flow rate, pH, and concentration of calcium, phosphorous, sodium, potassium, chloride, glucose and α-amylase. We, therefore, measured the evolution of these parameters throughout pregnancy.

METHOD: A cross-sectional study compared sialometric and sialochemical analyses of 30 pregnant women vs. 30 age-matched non-pregnant women, and a longitudinal study evaluated the pregnant women in the first and third trimester of pregnancy.

RESULTS: Pregnant women presented acidic non-stimulated saliva, but neutral stimulated saliva pH, and no relevant changes in salivary flow rate. Scialochemical analysis showed decreased calcium levels, increased phosphate levels, and a progressive decrease in glucose levels throughout pregnancy.

CONCLUSION: Pregnancy significantly changes the oral biochemical milieu, creating a favorable environment for the development of oral pathology, in particular, dental caries.

KEYWORDS: Saliva; pregnancy; pH; calcium; phosphorous; glucose

INTRODUCTION

Pregnancy entails a number of physiological changes apparently centered on the main objective of adapting the human body to the specific needs of the mother-fetus complex. The progressively increasing level of hormones, such as estrogen, progesterone and human chorionic gonadotropin, are the driving force behind these alterations; the results are reflected in hematological, genitourinary, cardiovascular, respiratory, gastrointestinal, endocrine, muscular and skeletal systems.1

The oral cavity environment is also altered due to these systemic physiological changes. The pregnancy-associated gingivitis is a well-known and common pathology, reflecting this condition.2,3 Some studies suggest a higher prevalence of caries rate among pregnant women compared to non-pregnant controls, and an increase in the decayed, missing and filled teeth (DMFT) index throughout pregnancy,4,5 but others found no increase in cariogenic activity during pregnancy.6 On the other hand, oral pathology may also severely interfere with pregnancy outcome; in pregnant women periodontal disease represents a risk factor for preterm birth and low birth weight babies.7,8

Some short-term changes in salivary flow rates, pH, buffering capacity, and biochemical composition, during pregnancy have been reported.3,6,9-15 Changes in salivary composition and flow rate may compromise the integrity of the soft and hard tissues in the oral cavity. Saliva provides calcium, phosphate and proteins and
forms a protective pellicle on the surface of the teeth, which acts as a source of antibacterial substances and buffers.\textsuperscript{16-18}

However, results reporting sialometric and sialochemical analysis during pregnancy are not consistent, and often contradictory. Table 1 summarizes a literature review regarding the effect of pregnancy on the biochemical parameters of saliva.

Table 1 - Summary of literature review on the effect of pregnancy on non-stimulated (NSS) and stimulated (SS) whole saliva parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Effect</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSS</td>
<td>= Rockenbach,\textsuperscript{12} Ozturk\textsuperscript{6}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Naveen\textsuperscript{12}</td>
<td></td>
</tr>
<tr>
<td>NSS</td>
<td>= Saluja,\textsuperscript{19} Laine\textsuperscript{20}</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>↑ Naveen\textsuperscript{12}</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>↓ Naveen,\textsuperscript{12} Rockenbach,\textsuperscript{13} Ozturk,\textsuperscript{6} Jain\textsuperscript{21}</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>= Rockenbach,\textsuperscript{13} Guidozzi,\textsuperscript{22} Salvolini 1998\textsuperscript{15}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Ozturk\textsuperscript{6}</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>↓ Ozturk,\textsuperscript{6} Bakhshi,\textsuperscript{23} Salvolini\textsuperscript{12}***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>= Rockenbach,\textsuperscript{13} Guidozzi,\textsuperscript{22} Salvolini 1998\textsuperscript{15}***</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>= Guidozzi\textsuperscript{22}</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>↓ Guidozzi\textsuperscript{22}</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>= Guidozzi\textsuperscript{22}</td>
<td></td>
</tr>
<tr>
<td>α-Amylase</td>
<td>↑ Abrao,\textsuperscript{24} Salvolini\textsuperscript{13}</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The understanding on how pregnancy alters the oral milieu is relevant to help clinicians to better adjust their preventive strategies in order to minimize oral pathology during pregnancy and post-delivery.

So, the aim of the present study was to compare the biochemical analysis of saliva regarding calcium, phosphorous, sodium, potassium, chloride, glucose, α-amylase, pH and flow rate between pregnant and non-pregnant women, as well as, to evaluate the evolution of these parameters throughout pregnancy. Our results show that pregnancy significantly changes the oral biochemical milieu, creating a favorable environment for the development of oral pathology.

### MATERIALS AND METHODS

The initial sample was composed of 30 women in the 1\textsuperscript{st} trimester of pregnancy, who attended the outpatient clinic of the Department of Obstetrics and Gynecology of the Arrabida Hospital, Porto, Portugal, for routine examination. After three withdrawals, due to changed hospital attendance, this group of pregnant women was re-evaluated in the 3\textsuperscript{rd} trimester of pregnancy. The initial control group comprised 30 non-pregnant women aged between 18 to 40 years old attending the same hospital on routine clinical examination. Five dropped out, not attending to the consultation appointment. Exclusion criteria were high-risk pregnancy, patients with less than 16 teeth, menopausal subjects, cigarette smoking, drug addiction, and subjects presenting compromising systemic or oral disease.

The research protocol was in compliance with the Helsinki Declaration and was approved by the ethics committees of the Faculty of Dental Medicine and the Arrabida Hospital, Porto, Portugal. An informed, free and clear consent was provided for, and signed by all participants. Confidentiality was guaranteed at the storage and processing stages of all information.

### Sialometric analysis

Saliva was collected for biochemical analysis in a quiet room between 8:00 to 12:00 AM to minimize circadian rhythm effects and at least 2h after eating, tooth brushing or mouth washing. To collect unstimulated whole saliva, the participants were asked to spit the saliva contained in the floor of the mouth into a sterile plastic container. Afterwards, the participants were instructed to chew paraffin pellets (Ivoclar Vivadent Inc., Amherst, NY, USA) for the collection of stimulated saliva, and asked to spit the accumulated saliva into a different sterile plastic container. The total amount of stimulated and non-stimulated saliva collected over a 5-min period was registered, and the salivary flow rates (ml/min) were calculated. The saliva was frozen directly at -80°C until analysis.

### Sialochemical analysis

The salivary pH was measured immediately after saliva collection using pH indicator paper (5.0-8.0, Duotest, Germany). Salivary biochemical parameters were quantified by an automatic analyser, Pentra C200 (Horiba ABX Diagnostics, Switzerland) as described previously.\textsuperscript{25}

### Data analysis

Categorical variables were described through relative frequencies (%) whereas continuous variables were described using mean ± standard deviation (SD). The following tests were applied as appropriate: Chi-squared independence test to analyse hypotheses regarding the categorical variables and independent and paired Student t-tests concerning continuous variables to compare independent and paired groups respectively. A significance level of p < 0.05 was adopted. The analysis was performed...
using the statistical analysis program SPSS® v.17.0 (Statistical Package for Social Sciences).

■ RESULTS

The mean age and literacy did not differ significantly between pregnant and non-pregnant groups (Table 2).

The results of sialometrical and sialochemical analysis are shown in Table 3. Stimulated salivary flow rate showed a slight increase from the first to the third trimester of pregnancy. No differences were observed between non-pregnant and pregnant women regarding stimulated and non-stimulated salivary flow rate as well as for non-stimulated saliva flow rate throughout pregnancy.

Compared to the non-pregnant, pregnant women presented more acidic non-stimulated whole saliva in both the first and third trimester. No differences were found between pregnant and non-pregnant women regarding stimulated saliva pH, and no significant differences were observed in non-stimulated and stimulated saliva pH from the first to the third trimester.

Salivary ionized calcium levels were lower in the first and third trimester of pregnancy compared to non-pregnant women. In spite of that, inorganic phosphate was higher in pregnant women compared to non-pregnant attaining statistically significance, only during the first trimester. No differences were observed throughout pregnancy regarding calcium and phosphate saliva levels.

Salivary sodium levels were significantly reduced, but only in the first trimester of pregnancy in comparison to non-pregnant women; we observed an increase in salivary sodium levels from the first to the third trimester of pregnancy, but with no statistical significance.

Salivary levels of potassium and chloride did not differ between pregnant and non-pregnant women, throughout all pregnancy.

Although α-amylase levels were twice as high in pregnant women vs. non-pregnant women, no statistically significant differences were found between non-pregnant and pregnant women, nor throughout pregnancy.

In comparison to non-pregnant women, glucose levels were progressively reduced throughout pregnancy; a high level of statistical significance was recorded in the third trimester compared to non-pregnant women.

Table 2 - Age and educational level of pregnant and non-pregnant women

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant</th>
<th>Pregnant</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>32.64 ± 4.48</td>
<td>32.26 ± 4.15</td>
<td>0.752</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td>0.386</td>
</tr>
<tr>
<td>Basic</td>
<td>4.0% (1)</td>
<td>0.0% (0)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>40.0% (10)</td>
<td>29.6% (8)</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>56.0% (14)</td>
<td>70.4% (19)</td>
<td></td>
</tr>
</tbody>
</table>

Results expressed in prevalence (%) or as mean ± SD. P-values were calculated using non-paired student’s t-test for age comparison and using Chi-square test (2 cell have expected count less than 5) for literacy comparison.

Table 3 - Biochemical analysis of saliva of non-pregnant (NP) and pregnant (P) women, in the first and third trimester of pregnancy (1st T and 3rd T)

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant</th>
<th>Pregnant</th>
<th>P-values</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary flow, mL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSS</td>
<td>1.75 ± 0.44</td>
<td>1.86 ± 0.49</td>
<td>0.409</td>
<td>0.247</td>
</tr>
<tr>
<td>SS</td>
<td>2.64 ± 0.31</td>
<td>2.55 ± 0.37</td>
<td>0.247</td>
<td>0.662</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSS</td>
<td>7.03 ± 0.26</td>
<td>6.69 ± 0.35</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SS</td>
<td>7.34 ± 0.29</td>
<td>7.33 ± 0.36</td>
<td>0.924</td>
<td>0.186</td>
</tr>
<tr>
<td>Calcium, mmol/L</td>
<td>0.51 ± 0.28</td>
<td>0.32 ± 0.28</td>
<td>0.022</td>
<td>0.013</td>
</tr>
<tr>
<td>Phosphate, mmol/L</td>
<td>4.45 ± 1.93</td>
<td>6.50 ± 3.67</td>
<td>0.040</td>
<td>0.081</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>11.68 ± 11.80</td>
<td>4.04 ± 4.66</td>
<td>0.006</td>
<td>0.894</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>20.12 ± 3.39</td>
<td>23.75 ± 6.89</td>
<td>0.073</td>
<td>0.191</td>
</tr>
<tr>
<td>Chloride, mmol/L</td>
<td>35.96 ± 15.29</td>
<td>41.23 ± 18.09</td>
<td>0.346</td>
<td>0.277</td>
</tr>
<tr>
<td>α-Amylase, U/L</td>
<td>117.82 ± 148.85</td>
<td>212.83 ± 416.92</td>
<td>0.292</td>
<td>0.172</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>3.29 ± 4.38</td>
<td>1.65 ± 2.39</td>
<td>0.57 ± 0.46</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Results expressed in mean ± SD. NSS, non-stimulated saliva; SS, stimulated saliva. P-values were calculated using non-paired student’s t-test for comparison between non-pregnant and pregnant women and paired student’s t-test for comparison between measurements at 1st and 3rd trimester in pregnant women.

■ DISCUSSION

Globally, our results show that pregnant women presented acidic non-stimulated saliva but neutral stimulated saliva pH, no relevant changes in salivary...
flow rate, decreased saliva calcium level, increased saliva phosphate level and a progressive decrease in saliva glucose level throughout pregnancy.

Major physiological changes may occur during pregnancy affecting the entire body, reaching far beyond the maternal-fetal complex. There are many studies evaluating pregnancy-induced physiological alterations, but few look specifically to the biochemical changes in the oral cavity. As noted in the introduction, results of these studies are not consistent and may be even contradictory.6,12,13,19,24

Among all the analysis performed, and taking together previous studies and the present study, we can confidently assume that pregnancy reduces non-stimulated saliva pH.6,12,13,19,21 The decrease of saliva pH during pregnancy may be partially justified by the higher number of daily meals.6,26 The acidic saliva throughout pregnancy represents a risk factor for dental caries development.27

On the other hand, stimulated saliva pH remains neutral throughout pregnancy. Our results corroborate the work of Saluja and colleagues.19 One can hypothesize that chewing gum maybe favourable to maintain oral pH neutral throughout pregnancy. Further studies are necessary to confirm this hypothesis. Interestingly, recent studies show that chewing gum induces no changes in gastric pH28,29 and is associated with early recovery of bowel motility and shorter length of hospital stay for women after caesarean section.30

Regarding the other parameters analysed in saliva, namely salivary flow rate, calcium, phosphate, sodium, potassium, and α-amylase, different results have been reported, and together with our study, no consensus has emerged. The differences in the reported values may be justified by normal intra-individual changes of saliva content that may be linked to the circadian rhythms, menstrual cycle, eating habits, therapeutic drugs, etc.31,32,33,34 Also, when comparing non-pregnant to pregnant women, the pregnancy period/trimester may also be critical, given that salivary biochemical changes were reported throughout pregnancy in our study and in some others.14,15,21,23,34,35

Regarding salivary flow rate, most studies, including ours, reported no changes between pregnant and non-pregnant women;6,13,19,20 however, Naveen et al.12 showed higher rates for either stimulated or non-stimulated saliva. In addition, reports regarding salivary flow rate throughout pregnancy are controversial,34,35 suggesting that many factors may regulate this flow during pregnancy.

When we look to our results regarding salivary calcium and phosphate levels, we see an interesting phenomena: in parallel to the 37% and 33% decreases in calcium levels in first and third trimester, respectively, we observe increases in phosphate levels of the same magnitude, 46% and 31% in first and third trimester, respectively. The formation and mineralization of teeth are greatly influenced by calcium and phosphate metabolism;36 accordingly, low calcium content in saliva has been associated to dental caries,37-39 due to enhanced enamel demineralization, reduced remineralisation and increased alveolar bone loss.37,39 It should be noted that higher salivary phosphate levels were found in children with early childhood caries.38 These inverted proportional calcium/phosphate saliva concentrations may be justified by phosphate homeostasis, where the secretion of parathyroid hormone in response to low serum calcium can increase phosphate efflux from bone, kidney and gastrointestinal tract.40

Pregnancy does not seem to alter salivary chloride levels and in this respect our results are concordant with a previous report by Guidozzi et al.22 This result is in agreement with the fact that plasma chloride levels are not altered during pregnancy.1 Regarding salivary potassium levels, we found that pregnant women did not differ from non-pregnant women, whereas results from Guidozzi and colleagues42 showed lower salivary potassium levels in saliva of women in the third trimester of pregnancy. If we hypothesize that salivary potassium levels may be correlated with plasma potassium levels, no changes should be expected in its blood levels during pregnancy.1

The main function of salivary α-amylase is the hydrolysis of starch into maltose; however, it can function as a substrate for oral bacteria, leading to the production of acids, which can promote enamel demineralization.41 Previous studies showed higher α-amylase levels in saliva during pregnancy,15,24 in parallel with higher blood levels.1 In our study, although α-amylase levels were higher in pregnant than in non-pregnant women, the difference did not attain statistical significance. The increased α-amylase levels in saliva during pregnancy may contribute to the acidic oral environment.

To our knowledge, this study was the first to compare salivary glucose levels between pregnant and non-pregnant women and to evaluate its progression throughout pregnancy. Interestingly, glucose levels were progressively reduced during the evolution of pregnancy. This result may be directly correlated to plasma glucose levels, given that during pregnancy increased insulin resistance is observed, with hyperinsulinaemia, and hypoglycemia during fasting.1 These changes are conditioned by hormones produced by the fetal-placental unit, and are intended to ensure an adequate supply of glucose to the fetus.

The worsening of periodontal disease during pregnancy is common and a known risk factor for preterm birth and low birth weight babies, due to the translocation of mediators of inflammation.7,8 The biochemical changes evaluated in our study do not seem to explain this phenomenon. Other well-known factors induced by the increased production of sex steroid hormones include increased gingival inflammation, increased gingival bleeding and crevicular fluid flow.42
CONCLUSION

In summary, pregnant women presented acidic non-stimulated saliva but neutral stimulated saliva pH, no relevant changes in salivary flow rate, decreased saliva calcium level, increased saliva phosphate level and a progressive decrease in saliva glucose level throughout pregnancy. These altered oral biochemical milieu conditions present favorable conditions for the development of oral pathologies, in particular caries.

ACKNOWLEDGMENTS

We thank Isabel Morais for the enriched discussion about pregnancy effects on oral and systemic milieu.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS


O EFEITO DA GRAVIDEZ SOBRE A BIOQUÍMICA SALIVAR

OBJETIVO: Os achados relativos às análises sialométricas e sialoquímicas durante a gravidez não são consistentes, e por vezes são mesmo contraditórios. Assim, fizemos uma revisão da literatura e comparamos os níveis salivares de cálcio, fósforo, sódio, cloreto, cloreto, fosfato, α-amilase, pH e a taxa de fluxo salivar entre mulheres grávidas e não grávidas, bem como, avaliamos a evolução desses parâmetros ao longo da gravidez.

MÉTODO: Realizamos um estudo transversal comparando a bioquímica salivar de um grupo inicial de 30 mulheres grávidas com um grupo inicial de 30 mulheres não gestantes da mesma idade seguido de um estudo longitudinal avaliando as mulheres grávidas no primeiro e terceiro trimestre de gravidez.

RESULTADO: As mulheres grávidas apresentaram um pH da saliva não estimulada ácido, mas um pH da saliva estimulada neutro, assim como diminuição dos níveis salivares de cálcio, aumento dos níveis salivares de fosfato, e uma diminuição progressiva nos níveis de glicose na saliva ao longo da gravidez.

CONCLUSÃO: A gravidez muda significativamente o ambiente bioquímico oral, criando condições favoráveis para o desenvolvimento de patologia oral, em particular da cárie dentária.

PALAVRAS-CHAVE: Saliva, gravidez, cálcio, fósforo, glicose

REFERENCES