Evaluation of alpha amylase and peroxidase in saliva of pregnant women

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Abstract: Background: Pregnancy is a natural physiological state that involves several biochemical modifications. Saliva is consisted of many types of proteins such as salivary alpha amylase and salivary peroxidase that might be affected by pregnancy. The former enzyme is considered one of the most prevalent proteins that is released by highly differentiated epithelial acinar cells and has been shown to have enzymatic activities while the latter has been approved that it has a significant role in oral health. The purpose of this study was to evaluate the salivary levels of alpha-amylase and peroxidase in pregnant and non-pregnant women. Materials and Methods: Sixty pregnant women were grouped according to the pregnancy trimesters. The first group involved 20 women in the 1st trimester, 2nd group represented by 20 women in the 2nd trimester while the 3rd group involved the rest who were in the 3rd trimester. In addition to 20 married non pregnant women as a control group. Salivary samples were collected from each group to compare the salivary level of alpha-amylase and peroxidase using sandwich enzyme-linked immune-sorbent assay. Results: 2nd and 3rd groups illustrated high significant level of these enzymes in comparison with the control group. However, the first group demonstrated non-significant differences in the level of tested enzymes when compared to that of the control group. Conclusion: It was concluded that the salivary alpha amylase and salivary peroxidase have higher levels in pregnant women with dramatic increase for those in the third trimester.

Keywords: Pregnancy, Salivary alpha amylase, Salivary peroxidase.

Introduction

Pregnancy is a unique moment in a woman’s life, with complicated physiological, anatomic and hormonal changes (1,2). Pregnancy hormones fluctuations may affect saliva composition (3).

Saliva is a fluid material generated in the human oral cavity that contains around 98 % water and the remaining 2 % glycoprotein, electrolytes and antibacterial components such as specific kinds of lysozyme enzyme and immunoglobulins (4). Saliva has several defense mechanisms, including locally and systemically generated enzymes, immunoglobulins, cytokines and a variety of antimicrobial peptides (AMPs)(5).

Maltose an important nutrient for bacteria is produced when starch is hydrolyzed by salivary alpha amylase and continous production of maltose as a nutrient for bacteria in oral cavity, may be an important factor in tooth decay production (6). In addition, alpha amylase also works in dissolving starch-in food remained after a meal by forming higher soluble components that may dissolve in saliva. Salivary alpha amylase not only facilitate the fermentation of bacterial carbohydrate and bacterial adhesion into tooth surfaces, also it binds selectively to oral bacteria (7).

Oral peroxidases are a component of the saliva’s innate non-immune defensive system (8). Peroxidases activate the oxidation of thiocyanate (SCN), a pseudohalide, in the oral medium, leading in hypothiocyanite (OSCN), a potent oxidant able to kill various bacterial species, fungi, viruses in vitro and mycoplasmas. The two most common peroxidases present in the mouth cavity are sialoperoxidase and myeloperoxidase. Myeloperoxidase high in the dento-gingival sulcus region, whereas sialoperoxidase
common in oral secretions and biofilms coated the oral surfaces (9). Oral peroxidases contribute in the protection of the tissues around the oral cavity against microbial damage by controlling the commensal oral flora and consuming bacterial H2O2, that consider harmful to the oral mucosa(10).

This study aimed to estimate salivary alpha amylase level in pregnant women in comparison to married non pregnant women and study the variations of these enzymes level in different trimester of pregnancy.

Materials and Methods

The current study (case-control study) was conducted from the month of December 2021 to the month of March 2022. The process of collecting samples was conducted from the Medical City / Baghdad Hospital / Women’s Consultant and also from Al-Mustansiriyah Health Center in Baghdad. Eighty women aged (20 – 30) years were enrolled in this study, 60 pregnant women represent (study groups) and divided into three groups, 20 from the first trimester, 20 from the second trimester, 20 from the third trimester and 20 married non-pregnant women represent the control group. Samples were collected after the approval had been obtained From the Scientific Research Ethics Committee with project number (383821), University of Baghdad. In addition, the subjects were given with complete information about the study’s purpose and they signed on completed informed consent before beginning.

Exclusion criteria
For both study and control groups, the exclusion criteria consisted of the followings:
• Presence of systemic disease (diabetes, hypertension, cardiovascular disease) that may affect oral health.
• Medications which may affect periodontal health condition.
• Use of anti-inflammatory and antibiotic drugs during the last month before examination.
• Smoking.
• History of abortion.
• Obesity.
• History of polycystic ovaries.
• Hormonal disturbances.
• Risk of inducing labor.
• Use of contraceptive.
• Wearing fixed or removable dental prostheses.

For both study and control groups, the permanent residence was within Baghdad city. Additionally, for the control group, the women were not examined during menstrual cycle.

Inclusion criteria
Healthy pregnant women.

Biomarker detection

At morning (9-11A.M) saliva was collected from each woman. Under standardized situation according to the instructions constructed by Navazesh(11), samples was taken from study groups and control group. four ml of saliva had been drawn in a test tube from each subject. After collecting the salivary samples, the tubes were placed in a cool box with ice and transferred to the laboratory. saliva sample were centrifuged for 10 minutes at 3000 rpm at 28°C. The supernatant was transported and save in tubes, numbering and freezing at -20°C until used for biomarkers detection procedure. Detection level of alpha amylase and salivary peroxidase was done by using commercially available enzyme-linked immune-sorbent assay (ELISA) kit based on sandwich enzyme-linked immune-sorbent assay technology and performed as recommended in leaflet with kit (MyBiosource; USA). The color change on microtiter plate of this kit was measured spectrophotometrically at a wavelength of 450nm ± 10nm.
Statistical analysis

Data description, analysis and presentation have been performed using computerized software statistical package for social science (SPSS version-22). The descriptive statistics included: minimum, maximum, mean, standard deviation (SD) and graphical presentation by bar charts, also used Tukey honestly significant difference (HSD)/post hoc test was used to determine if the relationship between two sets of data is statistically significant. Not significant P>0.05, Significant P<0.05.

Results

Level of salivary alpha amylase

Table (1) demonstrate the levels of salivary alpha amylase in all study and control groups, the results showed significant differences among all groups and the mean value of first trimester group was (67.20 ± 3.79), (74.39 ± 8.20) for second trimester group, (90.58 ± 5.54) for third trimester group and (58.32 ± 2.77) for control group.

<table>
<thead>
<tr>
<th>Salivary alpha-amylase (ng/ml)</th>
<th>Study groups</th>
<th>ANOVA (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First trimester (n=20)</td>
<td>Second trimester (n=20)</td>
</tr>
<tr>
<td>Minimum</td>
<td>59.41</td>
<td>60.01</td>
</tr>
<tr>
<td>Maximum</td>
<td>72.36</td>
<td>85.06</td>
</tr>
<tr>
<td>Mean</td>
<td>67.20</td>
<td>74.39</td>
</tr>
<tr>
<td>SD</td>
<td>3.79</td>
<td>8.20</td>
</tr>
</tbody>
</table>

[*] significant.

Comparison of salivary alpha-amylase level between subgroups.

Table (2) revealed the comparison between intergroup of salivary alpha amylase by using tukeys test and the results showed that there was significant differences and the mean difference was 7.18(0.0005) between first and second trimester, 23.37(0.0000) between first and third trimester, 8.89 (0.0000) between first trimester and control, 16.19(0.0000) between second and third trimester, 16.07(0.0000) between second trimester and control, 32.26(0.0000) between third trimester and control.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean difference</th>
<th>Tukey’s HSD (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>first trimester vs. second trimester</td>
<td>7.18</td>
<td>0.0005*</td>
</tr>
<tr>
<td>first trimester vs. third trimester</td>
<td>23.37</td>
<td>0.0000*</td>
</tr>
<tr>
<td>first trimester vs. control</td>
<td>8.89</td>
<td>0.0000*</td>
</tr>
<tr>
<td>second trimester vs. third trimester</td>
<td>16.19</td>
<td>0.0000*</td>
</tr>
<tr>
<td>second trimester vs. control</td>
<td>16.07</td>
<td>0.0000*</td>
</tr>
<tr>
<td>third trimester vs. control</td>
<td>32.26</td>
<td>0.0000*</td>
</tr>
</tbody>
</table>

[*] significant.

Level of salivary peroxidase

Table (3) demonstrated the levels of salivary peroxidase in four study groups, the results showed highly significant differences among all groups and the mean value of first trimester group was (14.80 ± 2.81), (18.70 ± 2.48) for second trimester group, (23.23 ± 4.55) for third trimester group and (13.08 ± 1.36) for control group.
Table 3: comparison in mean of salivary peroxidase among study and control groups.

<table>
<thead>
<tr>
<th>Salivary peroxidase (pg/ml)</th>
<th>Study groups</th>
<th>ANOVA (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>first trimester n=20</td>
<td>second trimester n=20</td>
</tr>
<tr>
<td>Minimum</td>
<td>10.61</td>
<td>13.19</td>
</tr>
<tr>
<td>Maximum</td>
<td>19.97</td>
<td>21.51</td>
</tr>
<tr>
<td>Mean</td>
<td>14.80</td>
<td>18.70</td>
</tr>
<tr>
<td>SD</td>
<td>2.81</td>
<td>2.48</td>
</tr>
</tbody>
</table>

[∗] significant.

Comparison of salivary peroxidase level between subgroups.

Table 4 showed the comparison between intergroup of salivary peroxidase by using tukeys test and the results revealed that there was significant differences and the mean difference between first and second trimester was 3.90 (0.0007), 8.43 (0.0000) between first and third trimester, 4.54 (0.0000 ) between second and third trimester, 5.61 (0.0000)between second trimester and control , 10.15 (0.0000) between third trimester and control ,while there was no significant difference between first trimester and control with mean difference was 1.71 (0.297).

Table 4: Inter group's comparisons of mean of salivary Peroxidase between all pairs of groups.

<table>
<thead>
<tr>
<th>Grouping</th>
<th>Mean difference</th>
<th>Tukey's HSD (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>first trimester vs. second trimester</td>
<td>3.90</td>
<td>0.0007∗</td>
</tr>
<tr>
<td>first trimester vs. third trimester</td>
<td>8.43</td>
<td>0.0000∗</td>
</tr>
<tr>
<td>first trimester vs. control</td>
<td>1.71</td>
<td>0.297NS</td>
</tr>
<tr>
<td>second trimester vs. third trimester</td>
<td>4.54</td>
<td>0.0000∗</td>
</tr>
<tr>
<td>second trimester vs. control</td>
<td>5.61</td>
<td>0.0000∗</td>
</tr>
<tr>
<td>third trimester vs. control</td>
<td>10.15</td>
<td>0.0000∗</td>
</tr>
</tbody>
</table>

[∗] significant.

Discussion

Salivary components, especially proteins, play a vital role in the antibacterial capacity of oral cavity’s (12). According to certain research, pregnant women have higher total salivary proteins than non-pregnant women; also, protein levels rise throughout the first to third trimesters of pregnancy (13). Alpha-amylase, is an important salivary proteins, that is mostly secreted, from parotid gland. The current study findings showed the mean value of salivary alpha-amylase was higher in study groups (pregnant women) in comparison to control group (married non pregnant women) with statically differences and the highest mean value appeared in third trimester followed by second trimester.

The findings of this study are supported by the findings of previous study (12,14,15) who discovered that pregnant women had greater levels of salivary alpha-amylase activity than non-pregnant women, with a considerable increase in alpha-amylase activity in the third trimester. On other hand some researchers contra verse these results and They reported that there was no significant change in alpha-amylase levels throughout pregnancy (16,17).

The present study’s findings might be attributed to psychological state of pregnant women. The rise in alpha-amylase levels during pregnancy might be attributed to sympathetic activity throughout physical and physiological stress, because pregnancy is a stressful state, salivary amylase levels rise. As stress in
pregnancy is not consistent salivary amylase rises as pregnancy progress. While Pregnant women who had other psychological stress will have more increasing salivary alpha amylase levels (15).

Also, salivary alpha amylase is a vital salivary protein that is mostly released by the parotid gland. The greatest important role of salivary alpha amylase is the dissolving of starch into maltose, and this product can be a substrate for bacteria in oral cavity and lead to acid generation, subsequently this lead to demineralization in dental enamel (6) and these effects may have an impact on oral health of pregnant women.

Oral peroxidase, is one of the most vital salivary antioxidant enzymes, is vulnerable to change as a result of numerous bodily conditions (18). The result of this current research showed a greater mean of salivary peroxidase in groups of pregnant women compared to non-pregnant women and the highest value was seen in third trimester followed by second trimester, these results are in accordance with the results of Laine (19) who discovered salivary peroxidase activity raised significantly through third trimester of pregnancy, supporting idea that this enzyme is oestrogen-dependent.

These results could be explained by the hormonal changes and variations during pregnancy like oestradiol that stimulates the metabolic activity of target tissues, leading to increased activity of peroxidase in the mammary glands and uterus (20), raised levels of various oral enzymes in pregnancy may also be clarified by the effect of oestrogen on epithelial, bacterial cells, and gingival tissue, with peroxidase being the sole enzyme whose activity followed oestrogen levels. The greatest peroxidase activity were seen through the pregnancy late stages, when the level of oestrogen peaked (21).

Limitations

The present study has some limitations, this include the limited sample size also several studies are needed in different places and on different race to support and confirm the findings of this study.

Conclusion

Salivary alpha amylase and salivary peroxidase levels increase during pregnancy and there is a variation in their levels according to trimester of pregnancy the highest values were recorded in third trimester.

Conflict of interest: The authors have disclosed no potential conflicts of interest.

Author contributions

AJJ; study conception, design, data collection and Methodology. ZAA and AJJ; statistical analysis and interpretation of results. AJJ and ZAA; original draft manuscript preparation. AJJ and FMA; Writing - review & editing. ZAA and FMA; Supervision. All authors reviewed the results and approved the final version of the manuscript to be published.

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Informed consent

Informed consent was obtained from all individuals or their guardians included in this study.
References


16. Braithwaite EC, Ramchandani PG, Lane TA, Murphy SE. Symptoms of prenatal depression are associated with raised salivary alpha-amylase levels. Psychoneuroendocrinology. 2015; 60:163-72. (Crossref).


العنوان: تقييم ألفا أميليز وبيروكسيداز في لعاب النساء الحوامل

الباحثون: الزهراء جابر جاسم، زينب الظاهر

المستخلص:
الخلفية: الحمل هو حالة طبيعية تتألف من العديد من العوامل الكيميائية الحيوية، بحيث تتضمن العديد من المركبات والجزيئات. إنزيم ألفا أميليز، وهو البروتين الأكثر انتشارًا في العضلات، يتم إنتاجه بواسطة خلايا الأميلاز الطفلي، وقد ثبت أن له أنشطة إنزيمية، وينتج عن الريبوزيم المركبات الكيميائية. إنزيم البيروكسيداز، وهو إنزيم يحتوي على دور محدد في صحة الفم، يتفاعل مع الجراثيم الفموية أثناء إزالة السموم من بيئة H2O2. يدمر هذا الأنزيم الجراثيم الفموية أثناء إزالة السموم من بيئة H2O2، مما يمنع نمو الجراثيم الفموية.

الهدف من هذه الدراسة هو تقييم انخفاض تواجد إنزيم ألفا أميليز والبيروكسيداز في النساء الحوامل بقيمة الممتزات المناعية وقياسها على إنزيمات الأنسجة المتواجدة في العوامل بالعظام ضيقة.

المستخلص:
النتائج: وجدت هذه الدراسة أنه هناك تزايد في تواجد إنزيم ألفا أميليز والبيروكسيداز في النساء الحوامل على مدى فترة الحمل، مع تزايد في تركيز إنزيمات الأنسجة المتواجدة في العوامل بالعظام ضيقة.

الخلاصة: إنزيمات ألفا أميلاز والبيروكسيداز تحوي على دور محدد في صحة الفم، وقد ثبت أن تزايد في تواجد إنزيمات الأنسجة المتواجدة في العوامل بالعظام ضيقة أثناء الحمل من المسببات المضادة للجراثيم الفموية.