

Research Article

# 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 1<sup>st</sup> trimester, 2<sup>nd</sup> group represented by 20 women in the 2<sup>nd</sup> trimester while the 3<sup>rd</sup> group involved the rest who were in the 3<sup>rd</sup> 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: 2<sup>nd</sup> and 3<sup>rd</sup> 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 <sup>(1,2)</sup>. Pregnancy hormones fluctuations may affect saliva composition <sup>(3)</sup>.

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 <sup>(4)</sup>. Saliva has several defense mechanisms, including locally and systemically generated enzymes, immunoglobulins, cytokines and a variety of antimicrobial peptides (AMPs)<sup>(5)</sup>.

Maltose an important nutrient for bacteria is produced when starch is hydrolyzed by salivary alpha amylase and continuous production of maltose as a nutrient for bacteria in oral cavity, may be an important factor in tooth decay production <sup>(6)</sup>. 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 <sup>(7)</sup>.

Oral peroxidases are a component of the saliva's innate non-immune defensive system <sup>(8)</sup>. 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<sup>(9)</sup>. 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 H<sub>2</sub>O<sub>2</sub>, that consider harmful to the oral mucosa<sup>(10)</sup>.

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<sup>(11)</sup>, 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 ( $67.20 \pm 3.79$ ), ( $74.39 \pm 8.20$ ) for second trimester group, ( $90.58 \pm 5.54$ ) for third trimester group and ( $58.32 \pm 2.77$ ) for control group.

**Table 1:** comparison in mean of salivary alpha amylase among study and control groups.

Salivary alpha-amylase ng/ml	Study groups				ANOVA (P-value)
	First trimester n=20	Second trimester n=20	Third trimester n=20	Control group n=20	
Minimum	59.41	60.01	72.94	54.34	
Maximum	72.36	85.06	97.01	61.83	0.0000*
Mean	67.20	74.39	90.58	58.32	
SD	3.79	8.20	5.54	2.77	

[\*] 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 2:** comparisons of salivary alpha-amylase between all pairs groups.

Grouping	Mean difference	Tukey's HSD (P-value)
first trimester vs. second trimester	7.18	0.0005*
first trimester vs. third trimester	23.37	0.0000*
first trimester vs. control	8.89	0.0000*
second trimester vs. third trimester	16.19	0.0000*
second trimester vs. control	16.07	0.0000*
third trimester vs. control	32.26	0.0000*

[\*] 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 ( $14.80 \pm 2.81$ ), ( $18.70 \pm 2.48$ ) for second trimester group, ( $23.23 \pm 4.55$ ) for third trimester group and ( $13.08 \pm 1.36$ ) for control group.

**Table 3:** comparison in mean of salivary peroxidase among study and control groups.

Salivary peroxidase pg/ml	Study groups				ANOVA (P-value)
	first trimester n=20	second trimester n=20	third trimester n=20	control group n=20	
Minimum	10.61	13.19	17.39	10.38	
Maximum	19.97	21.51	33.60	15.67	
Mean	14.80	18.70	23.23	13.08	0.0000*
SD	2.81	2.48	4.55	1.36	

[\*] 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.

Grouping	Mean difference	Tukey's HSD (P-value)
first trimester vs. second trimester	3.90	0.0007*
first trimester vs. third trimester	8.43	0.0000*
first trimester vs. control	1.71	0.297 <sup>NS</sup>
second trimester vs. third trimester	4.54	0.0000*
second trimester vs. control	5.61	0.0000*
third trimester vs. control	10.15	0.0000*

[\*] 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 <sup>(15)</sup>.

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 <sup>(6)</sup> 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 <sup>(18)</sup>. 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 <sup>(19)</sup> 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<sup>(20)</sup>. 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 <sup>(21)</sup>.

### **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

1. Mutlak NQ , Yas BA. Dental caries severity in relation to selected salivary variables among a group of pregnant women in Baghdad city/Iraq. J. Baghdad Coll. Dent. 2017;29(2):115-121. ([Crossref](#)).
2. Al-Najjar SN , Hussein B. Oxidative status among a group of pregnant women in relation to gingival health condition. J. Baghdad Coll. Dent.2019; 31(4). ([Crossref](#)).
3. Kivelä J., Laine M., Parkkila S., Rajaniemi H. Salivary carbonic anhydrase VI and its relation to salivary flow rate and buffer capacity in pregnant and non-pregnant women. Arch Oral Biol.2003; 48(8): 547-551. ([Crossref](#)).
4. Dawood IM., El-Samarrai SK .Saliva and Oral Health. Int. J. Adv. Res. Biol. Sci. 2018 ;5(7): 1-45. ([Crossref](#)).
5. Van 't Hof W., Veerman EC., Nieuw Amerongen AV., Ligtenberg AJ. Antimicrobial Defense Systems in Saliva. . Monogr Oral Sci 2014; 24:40-51. ([Crossref](#)).
6. Peyrot des Gachons C , Breslin PA. Salivary amylase: digestion and metabolic syndrome. Curr Diab Rep.2016;16(10):102. ([Crossref](#)).
7. Gong K, Mailloux L, Herzberg MC. Salivary film expresses a complex, macro molecular binding site for Streptococcus sanguis, J. Biol. Chem.2000; 275 (12): 8970–8974. ([Crossref](#)).
8. Bafort F, Parisi O, Perraudin JP, Jijakli MH. Mode of action of lactoperoxidase as related to its antimicrobial activity: A review. Enzyme Res. 2014;517164. ([Crossref](#)).
9. Ihalin R, Loimaranta V, Tenovuo J. Origin, structure, and biological activities of peroxidases in human saliva. Arch Biochem Biophys. 2006; 445(2):261-8. ([Crossref](#)).
10. Courtois P. Oral peroxidases: From antimicrobial agents to ecological actors (Review). Molecular Medicine Reports.2021; 24(1): 500. ([Crossref](#)).
11. Navazesh M ,Kumer SK. Measuring salivary flow. J. AM. D. Ass. 2008; 139: 35-40. ([Crossref](#)).
12. Rio R, Azevedo Á., Simões-Silva L., Marinho J., Silva MJ., Sampaio-Maia B. The biochemistry of saliva throughout pregnancy. Med Express. 2015;2(5).
13. Salvolini E, Di Giorgio R, Curatola A , Mazzanti L , Fratto G. Biochemical modifications of human whole saliva induced by pregnancy. Br J Obstet gynaecology.1998;105(6):656-60. ([Crossref](#)).
14. Guglielminotti J, Dehoux M, Mentré F, Bedairia E, Montravers P, Desmots JM, Longrois D.Int J Obstet Anesth. 2012 Jan;21(1):35-9. ([Crossref](#)).
15. Jaju JB, PraveenaVithpala AA, Doddi B, Poduri S. Salivary alpha amylase activity in pregnant and non-Pregnant females. Indian J Obst Gynecol Res. 2019; 6:165–168. ([Crossref](#)).
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](#)).
17. Shirzaiy M , Dalirsani Z. Comparison of Salivary Alpha-Amylase, Sialic Acid, and pH in Pregnant and Nonpregnant Subjects. Eur J Gen Dent 2021; 10:25–29. ([Crossref](#)).
18. Damirchi A, Kiani M , Jafarian V , Sariri R. Response of salivary peroxidase to exercise intensity. Eur J Appl Physiol. 2010;108(6):1233-7. ([Crossref](#)).
19. Laine M, Tenovuo J, Lehtonen OP, Ojanotko-Harri A , Vilja P, Tuohimaa P. Pregnancy-related changes in human whole saliva. Arch Oral Biol. 1988;33(12):913-917. ([Crossref](#)).
20. Rosado A , Delgado NM , Velazquez A , Aznar R , Martinez-Manautou J. Cyclic changes in salivary activity of N-acetyl-beta-d-glucosaminidase. Am J Obstet Gynecol. 1977;128(5):560-565. ([Crossref](#)).
21. Kumar P, Magon N. Hormones in pregnancy . Niger Med J. 2012 Oct-Dec; 53(4): 179–183. ([Crossref](#)).

**العنوان: تقييم ألفا أميليز وبيروكسيداز في لعاب النساء الحوامل**  
**الباحثون: الزهراء جابر جاسم 1 زينب الظاهر 2**  
**المستخلص:**

الخلفية: الحمل هو حالة فسيولوجية طبيعية تتضمن العديد من التعديلات الكيميائية الحيوية. يحتوي اللعاب على العديد من الجزيئات وتشمل هذه الجزيئات إنزيمًا مثل: (1) ألفا أميليز اللعابي وهو البروتين الأكثر انتشارًا الذي يتم إطلاقه بواسطة خلايا أسينار الظهارية شديدة التباين وقد ثبت أن له أنشطة إنزيمية (2) وقد ثبت أن بيروكسيداز اللعاب يحتوي على دور مهم في صحة الفم. ينظم هذا الإنزيم الجراثيم الفموية أثناء إزالة السموم من بيئته  $H_2O_2$  عن طريق أكسدة مجموعات الثيول. الغرض من هذه الدراسة هو تقييم ألفا أميليز اللعابي وبيروكسيداز اللعاب في النساء الحوامل وغير الحوامل. المواد والطرق: تم اختيار ستين من النساء الحوامل كمجموعات دراسة (20 في الفصل الأول ، و 20 في الثلث الثاني من الحمل ، و 20 في الثلث الثالث من الحمل) و 20 من النساء المتزوجات غير الحوامل كمجموعة ضابطة. تم أخذ عينة اللعاب من كل مجموعة لتقييم اللعاب **alpha-amylase** و **salivary peroxidase** باستخدام مقاييس الممتزات المناعية المرتبطة بإنزيم الساندويتش. النتائج: ظهرت نتيجة معنوية أعلى في المؤشرات الحيوية في المجموعات الحامل مقارنة بالمجموعات غير الحامل المتزوجة ، ولم يكن هناك فرق معنوي بين الثلث الأول من الحمل وبين المجموعة الضابطة. الخلاصة: ارتفاع مستويات ألفا أميليز اللعابية والبيروكسيداز اللعابي طوال فترة الحمل ، مع توثيق أعلى المستويات في الأشهر الثلاثة الأخيرة من الحمل.