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Comparison of Salivary Alpha-Amylase, Sialic Acid, and pH in Pregnant and Nonpregnant Subjects

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Abstract

Objectives During pregnancy, systemic physiological alterations lead to some changes in the oral cavity, which could prepare the mouth environment for oral and dental problems. This study was aimed to investigate salivary α-amylase, sialic acid levels, and pH levels in pregnant and nonpregnant females.

Materials and Methods In this analytical, case–control study, unstimulated saliva samples were collected with spiting method from 35 pregnant women (case group) and 35 nonpregnant women (control group) and transferred to the laboratory to assess salivary α-amylase, sialic acid, and pH levels. Data were analyzed by SPSS (version: 19) software through statistical methods of independent *t*-test and analysis of variance.

Results The mean sialic acid levels were 2.285 ± 1.230 mg/dL in pregnant and 2.744 ± 1.326 in nonpregnant women without any significant difference ($p = 0.138$). The mean salivary α-amylase concentrations were 2.461 ± 1.869 U/L and 2.439 ± 2.058 U/L, respectively, in pregnant and nonpregnant women, with no significant difference ($p = 0.963$).

The mean salivary pH in nonpregnant women was significantly more than that in pregnant women (7.845 ± 0.430 and 6.868 ± 0.413, respectively) ($p < 0.001$). Also, the mean salivary pH levels in pregnant women were 7.474 ± 0.420 in the first trimester, 6.868 ± 0.413 in the second trimester, and 6.568 ± 0.387 in the third trimester, which were significantly different ($p < 0.001$).

Conclusion Salivary sialic acid and α-amylase levels among pregnant women were no different from those of other subjects. During pregnancy, the salivary pH significantly reduced, and the mean salivary pH during pregnancy had a decreasing trend from the first trimester to the third trimester.

Keywords
- pregnancy
- saliva
- sialic acid
- α-amylase, alpha-amylases
- pH

Introduction

In pregnancy, physiologic changes cause the development of specific demands for both the pregnant woman and her fetus. Some changes, such as gingivitis, develop during pregnancy in the oral cavity, which could be related to hormonal alterations and unfavorable oral hygiene.[1,2]

Although some studies revealed a higher prevalence of dental caries and an increase in decay-missing-filled teeth (DMFT) in the pregnancy, other studies did not report any remarkable increase in the rate of caries.[3,4]

There is a theory mentioned that it is possible that some dental and oral problems might be related to the biochemical changes in the salivary composition, pH, and buffering

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capacity.[5-7] Salivary compositions including proteins and some ions such as calcium and phosphorous are important factors in the antimicrobial ability of oral cavity.[6]

Some studies showed that total salivary proteins in the pregnant women are more and calcium and phosphorous levels are less than nonpregnant ones; also, from the first to third trimesters of pregnancy, protein levels increase, and calcium and phosphorous fall.[7] Although, another study showed that concurrent with a reduction in pH and neutral capacity and calcium levels, an increase in phosphorous concentrations was observed during pregnancy.[6] Moreover, in the third trimester, the flow rate is more in pregnant women; however, pH and buffering capacity are less than nonpregnant subjects.[8] It seems that concurrent to elevated serum progesterone and estrogen in the pregnant saliva, pH and buffering capacity decrease.[8] These mentioned changes during pregnancy lead to more enamel demineralization and a decrease in the remineralization capacity of saliva.

Salivary α-amylase, which is the most important salivary protein, is secreted from the acinar cell of salivary glands thorough activation of adeno β-receptor 1.[9] The production of α-amylase in the salivary gland is independent of the salivary flow rate.[9,10] Because major salivary glands are innervated by parasympathetic and sympathetic nerves, measuring salivary proteins such as α-amylase could be a marker for evaluating autonomic activity.[9]

Some studies show that physical and psychological stress may cause an increase in salivary α-amylase,[11] since pregnancy is a physiologically stressful event, it could be effective on salivary composition.[9]

In respect to few studies that have been performed on salivary composition such as sialic acid, pH, and α-amylase, as well as the differences of some biomarkers in various races and populations, this study was aimed to assess these salivary markers in the pregnant subjects in Zahedan city.

Methods and Materials

Thirty-five pregnant (case group) and 35 nonpregnant women (control group), who were more than 20 years old, enrolled in this case-control study after signing informed consent forms.

A urine pregnancy test and ultrasonography were used for confirming pregnancy.

Nonpregnant subjects, who were in the follicular phase of the menstrual cycle and did not use oral contraceptive, enrolled as the control group. They were selected among the patients who were referred to Zahedan Dental School for routine dental treatments.

Inclusion criteria for both groups were: not having any systemic diseases (especially diabetes and hypertension), or mental or psychiatric problems, or periodontal or other chronic diseases or oral lesions; not smoking, no history of abortion; and not using any systemic drugs.

The check lists were filled out for all subjects, which included demographic data consisting of age, sex, and the week of pregnancy (for case group).

Saliva Collection

The subjects were asked not to eat, drink, smoke, or brush their teeth for 90 minutes before collecting saliva. First, all subjects washed their mouths with distilled water and after resting for 5 minutes, unstimulated saliva was collected by spitting method at 9 to 11 a.m.[12] After that, all women spat into 50 mL laboratory tubes every 60 seconds for 2 to 10 minutes. The salivary samples were stored with ice and sent to the laboratory, then the saliva samples were centrifuged at 2,500 *g* for 10 minutes and were stored at –20°C until analysis time.[13]

In the pregnant group, saliva samples were collected for sialic acid and α-amylase in the first trimester.

For the determination of sialic acid, a commercial sialic acid kit (Eastbiopharm Co, China) was used. The salivary samples were centrifuged at 800 *g* for 10 minutes. After washing the sediment with saline, it was centrifuged at 11,000 *g* for 5 minutes. After separation of saline supernatant fraction, thiobarbituric acid was added to the solution to complete this reaction, and sialic acid was assessed by the method of Shoza and Mphos.[14]

For the assessment of salivary amylase, commercial amylase kit (Bionik Diagnostic Systems, Iran) was used. The salivary α-amylase activity was evaluated using spectrophotometric assay. The amount of p-nitrophenol formation as a product of hydrolysis reaction with α-glucosidase was measured through the absorbance at the wavelength of 405 nm.[15]

For pregnant women, salivary samples were collected in three phases: the first trimester (9th week), the second trimester (20th week), and the third trimester (36th week).

Salivary pH was assessed by a digital pH meter (Electronics India). The pH meter was calibrated every day with prepared buffer solutions.

This study was approved by the ethical committee of Zahedan University of Medical Sciences (code: IR;ZAUMS.REC.1395.162).

The collected data was entered into SPSS software (version 19) and the results were statistically compared between two groups through *t*-test and analysis of variance (ANOVA) with a confidence interval of 95%. A significant level of 0.05 was set in this study.

Results

Thirty-five pregnant (mean age: 25 ± 4.8 years) and 35 nonpregnant (mean age: 23 ± 2.5 years) women were enrolled in this study.

Subjects' body mass index in the control group was between 20 and 20.9.

The pregnant subjects had an increase in weight of 10 to 12 kg during the pregnancy.

The Shapiro–Wilk test was used for assessment of normality of variables, and the distribution of the data was considered normal, if $p > 0.05$.

In the present study, distribution of all data was normal for every three variables. So, parametric tests such as *t*-test and ANOVA were selected for statistical analysis.

In the present study, there was not any significant difference between pregnant and nonpregnant women, according to salivary α-amylase and sialic acid (p = 0.963 and 0.138, respectively) (►**Table 1**).

As shown in ►**Table 1**, a significant different was observed between the two studies groups, according to salivary pH (p = 0.001).

Also, salivary pH significantly reduced in the pregnant subjects from the first to the third trimester (p = 0.001) (►**Table 2**).

Discussion

This study was designed to compare salivary α-amylase, sialic acid, and pH in pregnant and nonpregnant women.

There is a near correlation between saliva and plasma parameters. One of the most important salivary proteins is α-amylase, which is mostly secreted from the parotid gland. Previous studies showed various results. Bakhshi et al revealed an increase in unstimulated salivary total protein, and a decrease in calcium and phosphorous concentration[16]; however, the present study did not show any significant difference between the subjects according to one of the salivary enzymes, α-amylase.

Moreover, Abrao et al reported more salivary α-amylase during pregnancy compared with nonpregnant subjects,[17] although Rio et al similar to our study, did not find any remarkable difference in this biomarker between pregnant and other women.[6]

On the other side, Al–Nuaimy and Al–Doski demonstrated that in the pregnant women, salivary total protein, calcium concentration, and flow rate reduce, which is associated with an increase in gingival inflammation index and Decayed, Missing, and Filled Teeth (DMFT).[18]

The most important function of salivary amylase is hydrolysis of starch to maltose, which could act as a substance for oral bacteria and leads to acid production; consequently, this process accelerates demineralization of dental enamel.[19] It is confirmed that some factors could have an affect on secretion of α-amylase. In the present study, α-amylase did not any significantly change during pregnancy. The study done by Rio et al demonstrated an increase in salivary α-amylase during pregnancy.[6] However, there was no significant relationship between the amount and activity of α-amylase and the age of the pregnant women.[20,21] It is expressed that rising α-amylase in pregnancy is due to sympathetic activity during physical and physiological stress.[9] It seems that pregnancy depression leads to an increase in salivary α-amylase activity as compared to nondepressed pregnant women.[22]

History of early delivery, race, and women's temper during pregnancy could be reasons for contradictory results in the studies. Furthermore, psychological factors could affect secretion of some enzymes.[9,21] Giesbrecht et al believe that the sex of the fetus, race of mother, and pregnancy month is not related to daily α-amylase levels; however, history of miscarriage and trait anxiety, momentary depression, and positive temper causes an increase in α-amylase levels. Also, they expressed that chronic fatigue leads to a reduction in α-amylase. It confirmed that α-amylase is sensitive to emotional stimulation.[21]

The difference between the present study and other studies could be because of mothers' race and various laboratory methods for analyzing these biomarkers. In the present study, the subjects did not have any recognized psychiatric problems. Since temper problems have an affect on secretion of α-amylase, some different results are reasonable.

Also, in this study, there was no significant difference between pregnant women and other subjects according to sialic acid. Another study reported an increase in sialic acid during pregnancy.[23] Eliasson et al established that concurrent with rising estrogen after hormone therapy, salivary labial flow rate and buffering capacity increase that leads to a decrease in complaint of patients' dry mouth.[24] It is logical to claim that some salivary alteration occurs due to hormonal changes during pregnancy.

Salvolini et al reported an increase in sialic acid and a decrease in calcium and phosphorus at weeks 21th and 40th with an elevated α-amylase at weeks 10 and 21 of pregnancy. There is an opinion that concurrent with a decrease in salivary pH in the third trimester, sialic acid increases to improve bacterial clearance, in fact, sialic acid plays a protective role during pregnancy.[7]

In the present study, a decrease in salivary pH was detected during pregnancy, and there is a decreasing trend, from the first to the third trimester. Moreover, another study found a significant decrease in salivary pH in the third trimester as compared with the first trimester in pregnant women. Also, it was revealed that salivary pH during postpartum

Table 1 Comparison of salivary α-amylase, sialic acid, and pH between the case and control groups

Variables	Mean ± SD		*p*-Value[a]
	Pregnant	Nonpregnant	
Alpha-amylase (U/L)	2.46 ± 1.86	2.43 ± 2.05	0.963
Sialic acid (mg/dL)	2.28 ± 1.23	2.74 ± 1.32	0.138
pH	6.86 ± 0.41	7.84 ± 0.43	< 0.001

Abbreviation: SD, standard deviation.
[a]Independent *t*-test.

Table 2 Comparison of salivary pH between three trimesters in the pregnant women

Trimester	Salivary pH	*p*-Value[a]
	Mean ± SD	
First	7.47 ± 0.42	< 0.001
Second	6.86 ± 0.41	
Third	6.56 ± 0.38	

Abbreviation: SD, standard deviation.
[a]Repeated measures analysis of variance (ANOVA).

was lower than the control group.[25] Other studies found a decrease in pH and buffering capacity and an increase in gingival inflammation and DMFT during pregnancy.[18,26]

There may be a correlation between gingival inflammation and some alteration in cytokines such as the decrease in tumor necrosis factor-α levels in the third trimester.[27] Various reasons were mentioned for reducing pH, as well as decreasing buffering capacity in several studies.

Plasma bicarbonate and CO_2 of salivary glands are considered as the origins of salivary bicarbonate. During pregnancy, salivary protein composition changes, also progesterone causes a reduction in plasma bicarbonate, which leads to a decrease in pH and buffering capacity.[26] It is confirmed that the salivary flow rate and composition is affected by hormones, although its exact mechanism is not clear. Rising estrogen during pregnancy is concurrent with a reduction in sialic acid, pH, and buffering capacity.[26,28]

Naveen et al showed that salivary flow rate increases during pregnancy; however, pH and buffering capacity decrease in pregnant women as compared with nonpregnant subjects.[26] They mentioned that rising salivary flow rate could be due to an increase in estrogen and progesterone concentration. Moreover, reduced plasma HCO_3 and increased α-amylase might lead to decreased salivary pH and buffering capacity and prepare a suitable substrate for the acidogenic microorganisms.[26] The mentioned mechanism as well as an increase in number of daily meals and morning sickness contribute to an increase in susceptibility of pregnant women to dental caries.[5,28]

Rio et al analyzed biochemical composition of the saliva during pregnancy and demonstrated that unstimulated salivary pH was in the acidic range and stimulated salivary pH was in the neutral range. They revealed that pregnancy causes a decrease in calcium levels and an increase in phosphate levels.[6] Briefly, pregnancy changes oral biochemical environment and makes the mouth suitable for some oral damages such as dental caries.[6]

It is recommended to evaluate the correlation between salivary pH and other biomarker levels and oral and psychological alterations during pregnancy to reach comprehensive results for the oral status during pregnancy.

Conclusion

The present study showed that salivary α-amylase and sialic acid levels do not change; however, pH significantly decreases in pregnant women, and salivary pH has a reducing trend in the last months of pregnancy. Alteration in salivary composition could increase development of some oral conditions.

Conflict of Interest

The authors declare that they have no conflict of interest.

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