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Pregnancy related changes in human salivary secretion and composition in a Nigerian population

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Abstract

Background: A variety of physiological changes occurring during pregnancy has been shown to affect the oral health. Saliva is critical for preserving and maintaining the health of oral tissues and has been used as a source of non-invasive investigation of different conditions in human and animal studies.

Aim: This study was designed to evaluate changes in secretion and composition of saliva in pregnant women in a Nigerian population.

Methods: This was a descriptive cross-sectional study using purposive sampling technique. Saliva samples were collected from 50 pregnant and age matched 50 non-pregnant women. Salivary flow rate, pH, total protein and concentrations of sodium, potassium, calcium, phosphate and bicarbonate were determined and compared using paired independent sample t test.

Results: Salivary pH,mean concentrations of potassium and bicarbonate were significantly reduced while mean concentrations of salivary sodium and phosphate were significantly elevated in pregnant women compared to non-pregnant women ($P< 0.05$). However, there was no significant difference in the salivary flow rate, concentrations of total protein and calcium.

Conclusion: Salivary pH, bicarbonate and potassium concentrations were reduced while sodium and phosphate concentrations were elevated in pregnant women. These findings suggest that pregnant women may be predisposed to higher caries incidence.

Keywords: P*regnant women, non-pregnant women, salivary flow rate, salivary pH, total protein,*

Résumé

Contexte: Une variété de changements physiologiques qui se produisent pendant la grossesse a été montrée pour affecter la santé bucco-dentaire. La salive est critique pour la conservation et le maintien de la santé des tissus buccaux et a été utilisé comme source d'investigation non invasive de conditions différentes dans les études humaines et animales.

Objectif: Cette étude a été conçue pour évaluer les changements dans la sécrétion et la composition de la salive chez les femmes enceintes dans une population nigériane.

Méthodes: Ceci était une étude descriptive à cross-section utilisant la technique de l'échantillonnage raisonné. Des échantillons de salive ont été prélevés sur 50 femmes enceintes et appariés par 50 femmes non enceintes du même âge. Taux d'écoulement salivaire, le pH, protéines totales et les concentrations de sodium, potassium, calcium, phosphate et bicarbonate ont été déterminés et comparés en utilisant le test t d'échantillon indépendant apparié.

Résultats: pH salivaire, les concentrations moyennes de potassium et de bicarbonate ont été considérablement réduits tandis que les concentrations moyennes de sodium et de phosphate salivaires étaient significativement plus élevés chez les femmes enceintes par rapport aux femmes non-enceintes ($P <0,05$). Cependant, il n'y avait pas de différence significative dans le taux de flux salivaire, les concentrations de la protéine totale et de calcium.

Conclusion: pH salivaire, bicarbonate de potassium et concentrations ont été réduites tandis que les concentrations de sodium et de phosphate ont été élevés chez les femmes enceintes. Ces résultats suggèrent que les femmes enceintes peuvent être plus prédisposés à l'incidence de la carie.

Mots-clés: *femmes enceintes, femmes non enceintes, débit salivaire, pH salivaire, protéines totales*

Introduction

Saliva is a watery fluid produced by the major and minor salivary glands situated in the oral cavity and parts of the upper respiratory tract. Saliva consists of approximately 99% water and a variety of electrolytes and proteins. The components interact and are responsible for the various functions attributed to saliva [1]. Saliva is critical for preserving and maintaining the health of oral tissues and has been used as a source of non-invasive investigation of different conditions in human and animal studies [2-5]. The physiological functions of saliva include initial food digestion, taste perception, maintenance of tooth integrity, oral clearance, lubrication, and protection of the oral cavity against infections. The contribution of the salivary glands to the secretion and composition of saliva depends on various physiological factors including pregnancy, age, sex, time of the day, diet, exercise, type and

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intensity of stimulus as well as disease conditions like diabetes mellitus, Sjogrens syndrome and cystic fibrosis [1, 3, 6].

Pregnancy is a physiological process associated with many functional and compositional alterations in almost all systems of the body to varying extents. It is also a state of physiological stress which is accompanied by profound hormonal, biochemical and metabolic changes [7]. A variety of physiological changes occurring during pregnancy has been shown to affect the oral health [8]. Similarly, some studies in whites have shown that pregnancy alters the composition of saliva with varying results [9-11]. A study by Hugoson [9] reported an increase in total protein concentration of both stimulated and unstimulated parotid saliva in pregnant women whereas another study [7] reported a decrease in unstimulated whole saliva of pregnant women. In addition, Al-Nuaimy and Al-Dosky [7] reported decreased salivary flow rate and pH in pregnant women whereas another study [12] reported increase in the pH of both unstimulated and stimulated whole saliva of women in their late pregnancy and post-partum.These variations could be attributed to the different populations studied as well as different methods used by individual authors. However the relationship between pregnancy and salivary secretion and composition has been assessed by few studies leaving inconclusive information available. In addition, to the best of our knowledge no previous study has documented changes in salivary secretion and composition in pregnant black women more so that reference standards for normality are usually derived from data obtained in specific populations. Therefore this study aimed at assessment of salivary secretion and composition in pregnant and non-pregnant women in a Nigerian population.

Methods

Study design

This was a descriptive cross-sectional study using purposive sampling technique. The study received ethical clearance and approval by the Institution Research Ethics Committee (UI/EC/13/0069).

Study population

The study included 100 healthy human subjects (50 pregnant and 50 non-pregnantwomen; age and sex matched). The pregnant women were consecutive women attending the Antenatal Out Patients Department of a Maternity Teaching Hospital while the non-pregnant women were those attending the Family Planning Unit of same Hospital. Participants were provided information regarding risks and benefits of the study and consent was taken. Individuals with complications like hypertension and diabetes mellitus as well as those on oral contraceptives were excluded. In addition, individuals with history of gingival bleeding, tooth ache and intra oral swelling were excluded.

Bio data including, age, weight, height gestation age and last menstrual period of the participants were recorded using a self-administered proforma.

Saliva collection

Saliva collection was undertaken between 8am and 9am and participants had not had meal for at least 2 hours before collection. Whole saliva was collected by spitting method. Participants were asked to spit (after rinsing the mouth with distilled water) into calibrated universal plastic bottles placed on ice cubes for a period of 10 minutes. Rates of resting saliva secretions were expressed in mls/mins and the pH of saliva samples were determined using a calibrated digital pH meter. Volumes of the secretions were recorded and stored at -20°C until laboratory analysis.

Laboratory procedures

Saliva samples were defrosted at room temperature and then centrifuged at 6000 rpm for 10 minutes before being used for laboratory analysis in order to remove extrinsic contaminants such as oral epithelial cells, micro-organisms and food debris. Saliva samples were analyzed for the concentrations of K^+, Na^+, Ca^{2+}, Cl^-, PO_4^{2-} and HCO_3^{2-}. For the determination of salivary ions, saliva was diluted at either 1/100 or 1/1000 and K^+, Na^+ and Ca^{2+} concentrations were determined using flame emission spectrophotometry. Concentrations of Cl^- and HCO_3^{2-} were determined by Schales method using mercuric nitrate while concentrations of PO_4^{2-} was determined using Cyrus Fiske and Subbarow's method.Total protein concentration was determined using established colorimetric methods with the use of Helios spectrophotometer by reading samples at 720nm.Bovine serum albumin was used for calibration purposes.

Statistical analysis

The main outcome variables were mean values of salivary flow rate, pH, total proteins, sodium, potassium, calcium, chloride, bicarbonate and phosphate in pregnant and non-pregnant individuals. Data were analyzed using Independent-Samples T Test. The level of statistical significance was set at $p < 0.05$.

Results

Data are presented as mean ± standard deviation, ranges, with 95% confidence intervals for all variables assessed.

There were 100 participants comprising 50 pregnant and 50 non-pregnant women with a mean age of 31.12±5.54 years (range: 20 to 42 years). Mean age of the pregnant women was 29.64± 5.12 years (range; 20 to 42 years) while the mean age of non-pregnant women was 32.6± 5.59years (range 22 to 42 years). As expected, the mean Body Mass Index of the pregnant women (26.94 ± 4.46 kg/m^2) was higher than in non- pregnant women (23.32 ± 3.19 kg/m^2). However, there was no correlation between body mass index and salivary parameters in pregnant women (r = 0.05, P= 0.63).

Mean value of salivary flow rate was 0.43± 0.16 mls/min (range: 0.2 to 0.8 mls/mim) in pregnant women while it was 0.44± 0. 41 mls/min (range: 0.2 to 0.7 mls/min) in non-pregnant women. There was no significant difference in the salivary flow rate comparing pregnant and non-pregnant women (P= 0.79). Mean value of salivary pH was 6.37± 0.49(range: 5.2 to 7.2) in pregnant women while it was 6.99 ± 0. 61 mls/min (range: 5.8 to 7.9) in non-pregnant women. Salivary pH was significantly reduced in pregnant women compared to non-pregnant women (P= 0.001). However, there was no significant difference in the concentrations of salivary total protein and calcium comparing pregnant women with non-pregnant women (Table 1).

Mean concentration of salivary sodium was 23.69 ± 10.6mmol/L (range, 13 to 74mmol/L) in pregnant women while it was 7.16 ± 4.9 mmol/L (range, 0.3 to 23mmol/L) in non-pregnant women. Mean concentration of phosphate was 17.44 ± 5.42mmol/L (range, 9.1 to 31.3mmol/L) in pregnant women while it was 6.62 ± 2.64mmol/L (range, 0.5 to 10.5mmol/L) in non-pregnant women. Mean concentrations of salivary sodium and phosphate were significantly elevated while mean concentrations of salivary potassium and bicarbonate were significantly reduced (P< 0.005) in pregnant women compared to non-pregnant women (Table 2).

Table 1: Salivary flow rate, pH and Total protein concentration in pregnant and non-pregnant women

	Pregnant N = 50	Non-pregnant N = 50	95% Confidence Interval	P value
Flow rate (mls/min)	0.43 ± 0.16 (Range: 0.2 to 0.8)	0.44 ± 0. 41 (Range: 0.2 to 0.7)	-0.07 – 0.05	0.79
pH	6.37 ± 0.49 (Range: 5.2 to 7.2)	6.99 ± 0. 61 (Range: 5.8 to 7.9)	-0.84 – 0.4	0.00
Total protein (mg/dl)	0.39 ± 0.15 (Range: 0.1 to 0.7)	0.33 ± 0.32 (Range: 0.1 to 1.2)	0.04 – 0.16	0.22

Table 2: Concentrations of salivary electrolytes in pregnant and non-pregnant women

	Pregnant N = 50	Non-pregnant N = 50	95% Confidence Interval	P value
Sodium (Mmol/L)	23.69 ± 10.6 (Range, 13 to 74)	7.16 ± 4.9 (Range, 0.3 to 23)	13.25 – 19.81	0.000
Potassium (Mmol/L)	23.46 ± 4.9 (Range, 9.9 to 32.8)	28.17 ± 6.25 (Range, 18.7 to 39.8)	-6.93 – 2.47	0.000
Bicarbonate (Mmol/L)	3.89 ± 1.24 (Range, 1 to 8)	4.79 ± 1.16 (Range, 0.9 to 8.1)	-1.38 – 0.43	0.000
Phosphate (Mmol/L)	17.44 ± 5.42 (Range, 9.1 to 31.3)	6.62 ± 2.64 (Range, 0.5 to 10.5)	9.13 – 12.51	0.000
Calcium (mg/dl)	3.41 ± 1.78 (Range, 0.7 to 10.4)	2.77 ± 1.46 (Range, 0.1 to 7.1)	0.003 – 1.29	0.049

Discussion

Previous studies have shown varying results on the changes in salivary secretion and composition in

pregnancy [9-12]. Rockenbach *et al.* [11] reported no difference in the salivary flow rate, concentrations of calcium and phosphate comparing pregnant and non-pregnant women whereas Hugoson [9] showed reduced salivary flow rate as well as increased calcium concentration in pregnant women. These variations could be explained by differences in the study population, sampling procedures and physiologic variability of saliva. Gland-specific saliva can be used for analysis of changes specific to one of the major salivary glands, whereas, whole saliva represents the physiologic fluid in the mouth for the maintenance of oral health and is most frequently used for assessment of oral health under different conditions [13-15]. In addition, changes in whole saliva collected from the mouth may not necessarily follow those observed in a particular gland [16]. Furthermore, composition of saliva is different when it is produced spontaneously or after stimulation [17]. Although stimulated saliva is thought to be a measure of the functional capacity of the gland, unstimulated saliva is important for the maintenance of oral health, and unstimulated secretion predominates during sleep and most waking activities[18]. However, collection of true unstimulated saliva is difficult due to interferences of environmental stimuli, which may result in a wide range of salivary flow rates [11,19].

In the present study we evaluated unstimulated whole saliva in pregnant and non-pregnant women in a Nigerian population. Values of all the parameters were different from the normal reference range reported in whites [7, 11, 18]. Salivary pH, bicarbonate and potassium were reduced in pregnant women compared to non-pregnant women. Previous studies [7,11,18] have reported reduced salivary pH in pregnant women which is in agreement with our finding.The reduced pH of saliva in pregnant women could be attributed to many factors. Increased salivary amylase activity in pregnant women has been reported [9] which could induce a rise in the substrate for acidogenic micro-organisms, thus contributing to the decreased pH observed during pregnancy. In addition, the reduced salivary bicarbonate observed in this study could explain the reduced pH in pregnant women. Salivary bicarbonate is an important electrolyte that contribute to the pH of saliva, thus also affects the buffering effect of saliva [20]. Similar to our finding, Salvolini *et al.*(18) reported lower salivary bicarbonate level in pregnancy which was attributed to the effect of progesterone in lowering plasma bicarbonate concentration [21].The reduced salivary pH in pregnant women observed in this study could predispose pregnant women to higher incidence of caries.

Regarding salivary flow rate, there was no significant difference in the flow rate of saliva in pregnant and non-pregnant women. Similar to our finding, previous study [11] has reported lack of difference in the salivary flow rate in pregnant and non-pregnant women. On the other hand, Naveen *et al.* [22] reported an increase in the salivary flow rate in pregnant women whereas others [7, 9] showed decrease. Although, there was no difference in the salivary flow rate between pregnant and non-pregnant women, the concentration of potassium was reduced. This can be explained by the increased concentration of sodium due to the effect of estrogen and progesterone causing sodium retention. When sodium concentration of saliva is increased during secretion, the potassium level is decreased and vice versa [23].The variations in the results could be attributed to different study population and sampling techniques.

In this study, concentrations of salivary sodium and phosphate were higher in pregnant women compared to non-pregnant women. However, there was no difference in the concentrations of total protein and calcium when pregnant and non-pregnant women were compared. Elevated levels of sodium and phosphate in pregnant women observed in this study could be explained by the effects of hormones like estrogen and progesterone on the plasma levels of the electrolytes which could have contributed to the levels in saliva. The increased level of salivary sodium and phosphate in pregnant women could predispose them to higher calculus formation as well as gingival and periodontal diseases. Similar to our finding, Rochenbach *et al.* [11] reported no change in salivary concentrations of calcium in pregnant women. In contrary, other studies [7,18] reported decrease in salivary calcium in pregnant women which was attributed to the increased requirement of calcium by both mother and foetus. The lack of difference in the salivary total protein concentration in pregnant women compared to non-pregnant women observed in this study may be explained by the generally low level of total protein in saliva that does not usually correlate with plasma level [24].

Conclusion

Varying changes were observed in salivary secretion and composition of pregnant women. Salivary pH, bicarbonate and potassium concentrations were reduced while sodium and phosphate concentrations were elevated. These findings suggest that pregnant women may be predisposed to higher caries incidence.

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