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Full Length Research Paper

Evaluation of biochemical changes in unstimulated salivary, calcium, phosphorous and total protein during pregnancy

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Pregnancy is thought to be predisposed to the impairment of oral and dental health. As saliva contributes to oral homeostasis, this study aimed to compare the changes of total protein, calcium and phosphorous concentration in whole saliva between pregnant and non-pregnant Iranian women. Samples were composed of 60 pregnant and 60 non-pregnant women attending Mirza Koochak khan hospital in Tehran. Unstimulated whole saliva was collected to determine salivary protein, calcium and phosphorous concentration. Data were analyzed by SPSS 16 software, chi square and T-test. Total protein in saliva of pregnant women was significantly more than non pregnant women. Also, values of calcium and phosphorous were significantly lower in pregnant women than that of non pregnant group. While enhancing gestational weeks, salivary total protein increased and calcium and phosphorous levels reduced significantly. Pregnancy may alter biochemical composition of saliva and this could play an important role in the incidence of pregnancy-induced oral health changes.

Key words: Saliva, pregnancy, calcium, phosphorous, protein.

INTRODUCTION

Most clinical studies have shown that, pregnancy can enhance susceptibility to periodontal disease and dental caries (Laine, 2002; Kloetzel et al., 2011; Salvolini et al., 1998). Indeed, pregnancy induces an increased response of gingival tissue to local factors, such as plaque and calculus formation and this can be related to as biochemical changes in saliva (Sal volini et al., 1998).

Saliva is an important fluid in the oral cavity and plays an essential role in establishing oral health and function (Maria et al., 2006; Llena-Puy, 2006; Lawrence, 2002). Most researches have shown the advantage of using saliva in detecting physiologic or pathologic conditions because there is a close relationship between saliva and serum parameters (Llena-Puy, 2006; Lawrence, 2002; Sreebny, 2000; Luther et al., 2010; Ching et al., 2011; Sato et al., 2010; Gursoy et al., 2010). According to findings during pregnancy, saliva can can be affected by hormone level changes. Calcium, phosphorous and proteins in saliva are important components and any changes can affect teeth or oral health. Although, the effect of changes in sex hormones on dental carries is not completely known, it is thought that pregnancy causes changes in salivary biochemical markers (Laine, 2002).

The objective of this study was to compare these biochemical markers (protein, calcium and phosphorous) in unstimulated whole saliva in pregnant and non pregnant women.

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Table 1. Salivary calcium, phosphorous and total protein concentrations in pregnant and non-pregnant women.

Variable	Pregnant (N = 60)		Non-pregnant (N = 60)		P value
	Mean	SD	Mean	SD	
Calcium	1.3	0.8	2.7	0.9	P<0.001
Phosphorous	4.61	1.04	6.28	1.21	P<0.001
Protein	935	187.1	784	126.7	P<0.001

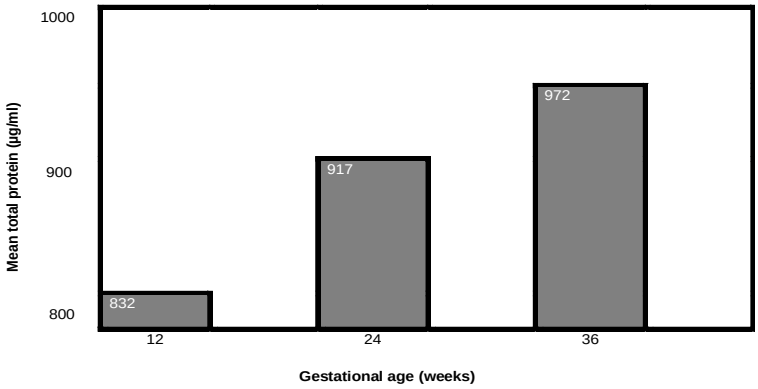

Figure 1. Whole salivary total protein content during pregnancy.

MATERIALS AND METHODS

This was a cross sectional study which was done among a sample composed of 120 women between 18 and 35 years old attending the Obstetrics and Gynecology Clinic, Mirza Koochak khan in Tehran. Three groups of 20 women of 12, 24, 36 weeks of gestation comprised the pregnant group. The comparison group consisted of 60 non-pregnant women. Mean age (26.5) was not significantly different between the pregnant and non-pregnant group. Exclusion criteria included systemic condition, drug or smoke consumption. Pregnant women were on their first pregnancy, had no history of abortion, their weight gain during pregnancy was in the normal limit (9 to 12 kg) and the blood pressure evaluation during the whole time was below 130/80 mm/Hg. Non-pregnant women attended their routine gynecology examination. All participants for the research were on proper diet and were given oral health instruction (brushing and flossing). All subjects signed an informed consent form before the study proceeded and its proposal was approved in Researches Committee of Qazvin University of Medical Science.

Sialochemical analysis

The collection of 2 ml unstimulated whole saliva was done under resting condition, between 09:30 am and 11:30 am, at least 1 h after eating and drinking. Following standard procedures, subjects were asked to wash their mouth and sit passively and expectorate in pre-weighed plastic containers for 5 min as the saliva accumulated in the floor of the mouth. The samples were frozen immediately after collection and subsequently transferred to the laboratory and stored at -20°C until sialochemical analysis. Saliva samples were centrifuged (centrifugal force: 3500 *g*) to remove bacteria and other extraneous material for 5 min. Total protein concentrations of samples were estimated by method of Bradford using bovine serum albumin as standard (Bradford, 1976). The salivary calcium concentration was determined by cresolphthalein complex (Calcium kit Darman Kave, Iran C.N: 10176).

The interaction of calcium with o-cresolphthalein complex one produces a red complex at alkaline pH with an absorbance maximum at 575 nm. The motivating color, measured at 575 nm is directly proportional to calcium concentration in the sample. The determination of inorganic phosphorous with ammonium molybdate and presence of sulphuric acid produces an unreduced phosphomolybdate complex (Phosphorousous kit ParsAzmun, Iran C.N: 88001-2).

The absorption of this complex at 340 nm is directly proportional to the inorganic phosphorous concentration (Fraser et al., 1986).

Statistical analysis

Data were analyzed by SPSS-16. software (Chicago, USA) and the comparison between the pregnant and non pregnant groups were performed using T-test and chi square. Values of P<0.05 were considered significant.

RESULTS

Pregnant women had higher total protein (935 µg/ml) than non pregnant women (784 µg/ml) but lower calcium (1.3 mg/dl) and phosphorous (4.61 mg/dl) than the latter (2.7 and 6.28 mg/dl) (P<0.001), respectively (Table 1).

Evaluating total protein content for 12,24 and 36 weeks of gestation showed a higher level in 36 weeks as compared to 24 and 12 weeks [832 µg/milt (SD = 152.1), 917 µg/milt (SD = 119.6), 972 µg/milt (SD = 121.3)], respectively. There were statistically significant differences between samples at 12, 24 and 36 weeks (P<0.05) (Figure 1).

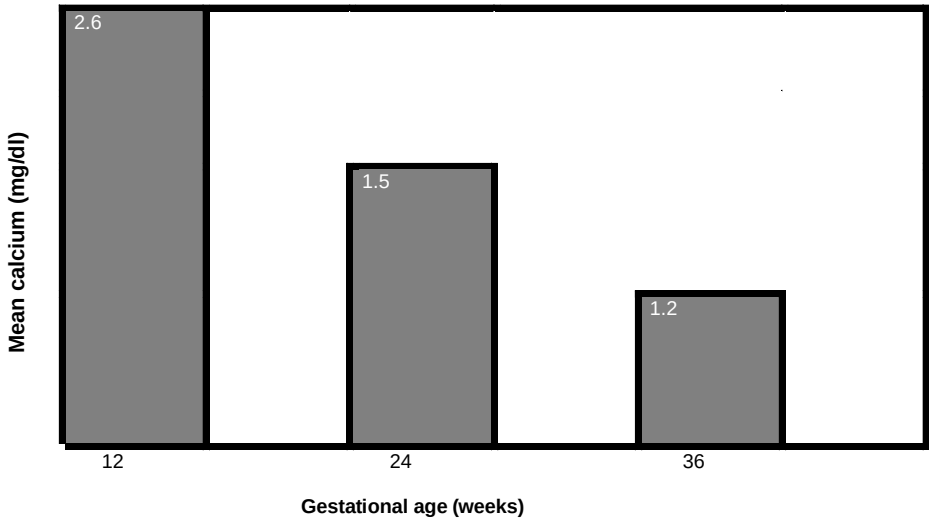

Figure 2. Whole salivary calcium concentration during pregnancy.

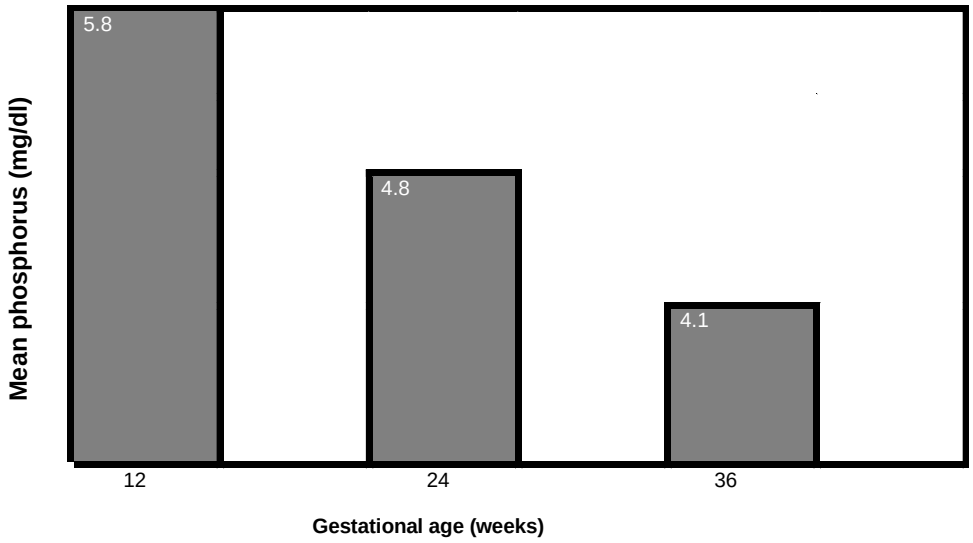

Figure 3. Whole salivary phosphorous concentration during pregnancy.

The salivary calcium concentration during pregnancy was lower at 36 weeks (1.18 mg/dl ± 0.8 Eu) than at 24 and 12 weeks (1.52 mg/dl ± 0.7 Eu; 2.63 mg/dl ± 0.6 Eu;) respectively. The values were significantly different between 12, 24 and 36 weeks ($P<0.05$) (Figure 2).

For phosphorous, saliva collected at 12, 24 and 36 weeks of gestation demonstrated a lower level in 36 weeks when compared with saliva phosphorous concentration from 12 and 24 weeks [5.82 mg/dl ± 1.08 Eu (12 weeks), 4.76 mg/dl ± 1.32 Eu (24 weeks), 4.1 mg/dl ± 1.12 Eu (36 weeks)].

The differences were statistically significant between the 12, 24 and 36 weeks, respectively ($P<0.05$) (Figure 3).

DISCUSSION

Saliva has multi potential effects on oral cavity such as lubrication, anti microbial effect, buffering, pH regulation and protection of teeth (Salvolini et al., 1998; Lawrence, 2002). Biochemical changes in saliva can lead to oral and dental tissue injury (Kloetzelet al., 2011; Salvolini et al., 1998). In these samples, salivary calcium, phosphorous and protein concentration were different, between pregnant and non pregnant Iranians women. Also, significant difference was found for pregnant women in different weeks of gestation. We decided to collect unstimulated whole saliva because this type of saliva predominates during most part of the day and is

important for maintenance of oral health, reflecting the physiological status of the oral cavity and the entire body (Little and Falace, 2008). Furthermore, pregnant women were not interested in collecting stimulated saliva. Most similar studies used unstimulated saliva too. We found higher average amount of protein concentration in pregnant women and there was a significant increasing protein concentration in correlation with weeks of gestation.

Salvolini et al. (1998) found higher total protein level in saliva of pregnant women than non pregnant women. Hugoson (1972) showed increased total protein in saliva from both parotid gland and whole saliva. Salivary protein can affect microbe's accumulation, adherence and nutrition therefore, higher protein concentration in pregnant women's saliva may result in dental or oral disease (Kloetzel et al., 2011). Conversely, Alessandro et al. (1989) showed lower total protein level in parotid gland in pregnant women than non pregnant women. Perhaps, the difference between the results is due to stimulated and unstimulated saliva composition. Sampling methods, race, nutrition, geographic and cultural differences are considered.

In our study, calcium, phosphorous levels were lower in pregnant women than in non pregnant women. Indeed, there was also a decrease in amount of Ca, and P in weeks of gestation during pregnancy. These results are in correlation with those of Salvolini et al. (1998). Hugoson (1972) showed an increase in the potassium and calcium level in stimulated and unstimulated saliva in pregnant women at first trimester and then a decrease in potassium level. Calcium ion in saliva helps to balance hard dental tissue and therefore reduction in its concentration during pregnancy may increase caries (Laine, 2002). Enamel has been mainly formed by hydroxyl apatite (calcium and phosphorous); saliva cause enamel tooth maturation after eruption of teeth as well as protecting it in oral cavity (James and Falace, 2008). In addition to create possibility of oral tissue damage during pregnancy, corresponding increase was found between weeks of gestation (Sreebny, 2000). Although, we studied total protein, the amount of separated proteins such as amylase, lysosym, peroxidase and lactoferrin reflects body's biochemical status and changes during pregnancy (Gursoy et al., 2010). The most important protein of saliva is α-amylase which is secreted by parotid gland. Increasing trend of this enzyme activity may lead to increase microorganism substitution and reduced pH saliva during pregnancy (Sreebny, 2000). Ciejak et al. (2007) found no relation between amount of salivary protein concentration and weeks of gestation. Relationship between salivary amylase activity and pregnancy was observed. Ciejak et al.(2007) found that α-amylase activity rate was lower in non pregnant women than pregnant women in 10 and 21 weeks of gestation. In addition to protein, calcium and phosphorous of saliva, other factors including IgA, pH and flow rate of saliva may play a role in oral health and changes may affect oral cavity status (Widerström and Bratthall, 1984). Our study suggests the evaluation of the earlier mentioned markers and their effect on saliva in future investigations.

Conclusion

This study provides further evidences for the changes in whole saliva composition during pregnancy. This could play an important role in pregnancy-induced dental caries or periodontal diseases.

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REFERENCES

Bradford M M (1976).A rapid and sensitive method for the quantitative of microgram quantities of protein utilizing the principal of protein-dye binding. Anal biochem. 72: 248-254.

Ching KH, Burbelo PD, Gonzalez-Begne M, Roberts ME, Coca A, Sanz I, Iadarola MJ (2011).Salivary anti-Ro60 and anti-Ro52 Antibody Profiles to Diagnose Sjögren's Syndrome .J Dent Res. 90(4): 445-449.

Ciejak M, Olszewska M, Jakubowska K, Zebiełowicz D, Safranow K, Chlubek D(2007).Activity of alpha-amylase and concentration of protein in saliva of pregnant women. Ann Acad .Med Stetin. 53: 42-45.

D'Alessandro S, Curbelo HM, Tumilasci OR, Tessler JA, Houssay AB(1989). Changes in hunan parotid salivary protein and sialic acid levels during pregnancy.Arch Oral Biol. 34: 829-31.

Fraser D, jones G, Kooh SW, Radde IC (1986).Calcium and phosphorous metabolism.In:Tietz NW,editor.Textbook of clinical chemistry.Philadelphia:W.B. saunders company, pp.1317-1372.

Gursoy UK, Könönen E, Pradhan-Palikhe P, Tervahartiala T, Pussinen PJ, Suominen-Taipale L, Sorsa T (2010).Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis .J Clin Periodontol. 37(6) :487-493.

Hugoson A(1972) .Salivary Secretion in Pregnancy a longitudinal study of flow Rate,total protein,sodium.Potassium and Calcium concentration in parotid saliva from pregnant women.Acta Odontol Scand. 30: 49-66.

Kloetzel M , Huebner CE, Milgrom P (2011) .Referrals for dental care during pregnancy.J Midwifery Womens Health. 5: 110-117.

Laine MA(2002).Effect of pregnancy on periodontal and dental health. Acta Odontol Scand. 60: 257-264.

Lawrence H. P (2002). Salivary markers of systemic disease: noninvasive diagnosis of disease and monitoring of general health .J Can. Dent .Assoc. 68(3): 170-174.

Little J ,Falace D (2008). Dental management of medically compromised patients. Canada, mosby. pp. 268-278.

Llena-Puy C(2006) .The rôle of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol. Oral Cir Bucal. 11: 449-455.

Luther T, Carrion CF, Cobb N, Le G, Edwards C, Schwartz S, Streckfus C (2010.) Methods for analyzing saliva proteins for systemic disease detection. Gen Dent. 58(2): 110-113.

Maria I. R, Sandra A. M, Elaine B .V, Laura L , Rosemary S(2006). Salivary flow rate, pH, and concentrations of calcium, phosphate, and sIgA in Brazilian pregnant and non-pregnant women. Head & Face Medicine. 2: 44.

Salvolini E, Giorgio R.Di , Curatola A, Mazzanti L,Fratto G(1998). Biochemical modifications of human whole saliva induced by pregnancy.Br. J. Obstet Gynaecol. 105: 656-660.
Sato J, Goto J, Murata T, Kitamori S, Yamazaki Y, Satoh A, Kitagawa Y (2010) .Changes in saliva interleukin-6 levels in patients with oral squamous cell carcinoma. Oral Surg. Oral Med .Oral Pathol .Oral Radiol Endod. 110(3): 330-336.
Sreebny L.M(2000).Saliva in health and disease: an appraisal and update.Int Dent J. 50(3): 140-161.
Widerström L, Bratthall D(1984).Increased IgA levels in saliva during pregnancy.Scand. J .Dent Res . 92(1): 33-37.