ORIGINAL ARTICLE

Salivary and Dental - Oral Hygiene Parameters in 3rd Trimester of Pregnancy and Early Lactation: The Effect of Education

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Abstract

To investigate the effect of 3rd trimester of pregnancy and lactation on dental, oral hygiene and salivary parameters according to educational status. Fourteen healthy women at their 3rd trimester and lactation periods, and 12 non-pregnant women as age-matched controls were recruited. Sulcus bleeding (SBI), plaque (PI) and caries (DMF-T) indexes were estimated and unstimulated whole saliva were taken for free calcium (Ca), inorganic phosphate (Pi), glutathione (GSH),total protein (TP) levels, carbonic anhydrase activity (CA), buffering capacity (BC), flow rate (FR) and pH determinations. Pregnant subjects were also sub-divided as high and low educated sub-groups. DMFT, SBI and PI increased significantly in lactation compared to pregnancy, pH and BC in pregnancy were significantly lower than non-pregnant condition and lactation. Ca and Ca/Pi molar ratio increased and Pi decreased significantly in pregnancy compared to non-pregnant condition. DMF-T, SBI and PI increased; FR and CA decreased significantly in low educated sub-group and SBI and Ca/Pi molar ratio decreased; pH and BC increased significantly in high educated sub-group in lactation compared to pregnancy. Within the limitation of our sub-groups sample size, daily oral hygiene care and good nutrition seem to have an effective impact on maintaining and/or improving them between pregnancy and lactation.

Keywords: Salivary parameters, DMFT, SBI, PI, pregnancy, lactation, education

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Introduction

Dental caries can be defined as a diet and saliva modified bacterial disease (1). The multiple functions of saliva play a significant role in the prevention of dental caries (2). Recent studies have reported dental caries to be more prevalent among pregnant women than non-pregnant controls and DMFT index to increase significantly during pregnancy (3. 4). Streptococcus mutans counts in maternal saliva have been well established and high levels have been reported (5-7).

Of the many pregnancy salivary studies, none have taken oral health parameters into account (7-9), Furthermore, most of them have been cross-sectional and have reported conflicting findings (7-9), which may have been due to different sampling methods i.e. saliva being stimulated or unstimulated or due to different saliva type i.e. total saliva or saliva from specific salivary glands. For oral health investigations, unstimulated whole saliva is the most representative factor since it bathes the oral cavity for more than 90% of the day (10). Also, unstimulated whole saliva is suitable for investigating salivary gland functions in female subjects (11, 12).

It has been suggested that the higher caries rates observed during pregnancy are influenced by changes in the biochemical composition of saliva and deterioration of oral hygiene due to food cravings, aversions (vomiting, nausea, reflux) due to increases in female sex hormones (13). Common salivary findings in the literature are that pH and buffering capacity (BC) decrease during pregnancy although flow rate (FR) does not change. Salivary cariogenic microbiota levels are at their highest during the 3rd trimester of pregnancy and lactation (7).

Since salivary parameters such as pH, BC, FR, glutathione (GSH), carbonic anhidrase activity (CA), free calcium (Ca) and inorganic phosphate (Pi) are known to be related with dental and oral hygiene parameters (1, 14-16), we aimed to investigate the effect of 3rd trimester of pregnancy and early lactation on these parameters in addition on dental and oral hygiene parameters, taking educational status into account.

Materials and Methods

Study Subjects and Design

The subjects of present study were consisted of 14 healthy pregnant women aged between 23 to 37 years (mean age; 29±3.9) and 12 non-pregnant women aged between 23 to 36 years (mean age; 28.6±3.8) as age-matched controls. The pregnant women were examined during their 3rd trimester of pregnancy (at 30-34 weeks) and later during the early lactation period (at 6-8 weeks). The pregnant women were sub-divided into two groups (n=7 per group) on the basis of the educational status as high educated and low educated. The mean ages of them 31.3 ± 2.9 and 26.7 ± 3.4 respectively.

Gestational age in weeks was taken as the time since the end of the last menstrual period and confirmed by ultrasound measurement of the crown–rump length. The non-pregnant women were in the early follicular period and the lactating women were not yet menstruating. All of the pregnant women subsequently had healthy babies within standard birth weight ranges in the 38th or 42nd week of their pregnancy.

Inclusion criteria for both the pregnant and non-pregnant women were as follows: no drug abuse, no consumption of alcohol or tobacco products, having no oral complaints, no previous medical abortion, having a history of regular menstrual cycles (28–30 days), no systemic diseases and no regular medication (except for supplemental doses of folic acid which they all had received), Exclusion criteria were a risk of inducing labor or unwillingness to participate in the study.

The study was designed in accordance with the guidelines issued in the Declaration of Helsinki. Written informed consent was provided by all participants.

Clinical Examination

The oral conditions of all subjects were recorded by the same experienced dentist. All fully erupted permanent teeth were scored except for third molars. The examiner used a mouth mirror. a blunt explorer and a manual periodontal probe in order to assess each woman's caries and periodontal status, recording all visible cavities and pre-cavities (17). The subjects received no professional dental, periodontal or oral hygiene treatmentor substances during the study. All subjects were instructed to maintain the regular dental hygiene practice throughout the study. Socio-demographic data were collected via a questionnaire.

DMF-T (Decayed, missing and filled teeth) indices were scored in accordance with World Health Organisation (WHO) criteria (18). The Sulcus Bleeding Index (SBI) was obtained by the method of Muhlemann and Son and the Plaque Index (PI) by that of Löe and Sillness (19).

Saliva Collection

Two hours prior to the saliva collection, all subjects were instructed not to eat anything after a standard breakfast. Unstimulated whole saliva samples were collected for 5 min from each subject at the same time of day (between 08.00 a.m. and 10.00 a.m.). During the saliva collection, the participants sat in a relaxed position with their heads bent forward, allowing the saliva to drain through the open lips into a test tube via a funnel. Immediately after collection, the saliva volume was measured and then the salivary flow rate was calculated in ml/min. Saliva samples were stored at -24 °C until used.

Salivary Analysis

The saliva samples were thawed and kept at 4°C. They were then centrifuged and the supernatant taken for analysis. Total protein (TP) level was determined by the method of Lowry (20), using bovine serum albumin as a standard, reading absorbance at 500 nm and expressing the TP level in g protein L^{-1}.

Salivary pH was directly measured with pH paper (pH indikatörpapier, Merck Neutrolit-5.5-9.0, Germany). A pH strip was dipped into the saliva and the color change on the strip used to estimate the pH. Salivary BC was measured by Ericsson's method (21) which is still used in dental research and is still a gold standard for the assessment BC (10, 22, 23). It is involved mixing 100 ml of saliva with 300 ml HCl (0.0033 M) and measuring the pH of the mixture with pH paper after 10 min.

Salivary free Ca and P_i were measured by BIOASSAY system kits [QuantiChromTM DICA-500 and DIPI-500 respectively] and values expressed as mM Ca or P_i.

The GSH levels were determined according to Beutler's method using Ellman's reagent (24). The SH groups reduce Ellman's reagent to form 5.5′-dithiobis 2-nitrobenzoic acid and the resulting color change is measured spectrophotometrically at 412 nm. Values were expressed as μM GSH.

CA activity was quantified on the basis of its esterase activity which was obtained in accordance with the method described by Verpoorte et al. (25). This involved recording the change of p-nitrophenylacetate to p-nitrophenylate ions over a period of 3 min at room temperature as indicated by its absorbance at 348 nm measured with a spectrophotometer. Blank runs in the absence of enzyme provided values for non-enzymatic hydrolysis rates. Values were expressed as units per ml saliva.

Statistics

Statistical analysis was conducted using Mann Whitney U and paired and unpaired t-tests. Pearson and Spearman correlation analysis was also performed (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL., USA). Relationships yielding p values less than 0.05 were considered to be significant.

Results

There were no significant differences in socio-demographic data between pregnant women and the aged-matched non-pregnant control women (Table1). The salivary pH and BC levels of the pregnant women were significantly lower than those of non-pregnant and lactating women. Ca and Ca/P_i molar ratio were significantly higher and P_i significantly lower in the pregnant group than in the non-pregnant group. The other salivary parameters were not significantly different between the groups (Table 2). The DMFT, SBI and PI indices were significantly higher in the lactating women than those in the pregnancy period (Table 2). Salivary pH positively correlated with TP and negatively with CA; salivary GSH negatively correlated with DMFT in the pregnant women (Table 3). DMFT value positively correlated with SBI and SBI with PI in both the pregnant and lactation periods (Table 3). Significant positive correlations were found between oral hygiene and educational levels and between occupation and nutritional status in all three groups (Table 3).

Table 1. Characteristics of the pregnant and non-pregnant women

	Pregnant (n=14)	on-pregnant (n=12)	*p value*
Age (mean±SD.Years) *	29±3.9	28.6±3.8	0.784
Education [n(%)]			
PhD in Dentistry	7 (50)	7 (58.3)	
High school	3 (21.4)	2 (16.7)	
Primary school	4 (28.6)	3 (25)	0.705
Occupation [n(%)]			
House wife	7 (50)	2 (16.7)	
Employee	7 (50)	10 (83.3)	0.123
Socioeconomic statu [n(%)]			
High	8 (57.1)	7 (58.3)	
Middle	1 (7.1)	3 (25.0)	
Low	2 (14.3)	2 (16.7)	0.595
Very low	3 (21.4)	0 (0)	
Number of children [n(%)]			
Having no child	9 (64.3)	6 (50)	
Having one child	4 (28.6)	6 (50)	
Having 2 children	1 (7.1)	0 (0)	0.667
Tootbrushing [n(%)]			
Twice a day	7 (50)	6 (50)	
Once a day	1 (7.1)	2 (16.7)	
Once a month	3 (21.4)	2 (16.6)	0.860
Never	3 (21.5)	2 (16.7)	
Nutrition [n(%)]	7 (50)	6 (50)	
0	2 (14.3)	3 (25)	
1	5 (35.7)	3 (25)	
2			0.820

Mann-Whitney U results except Age (*t-test results). **Nutrition;** 0: Regular diet with supported animal and vegetable foods. 1: Carbohydrate-based diet supported with animal and vegetable foods. 2: Carbohydrate-based diet. **Socioeconomic status;** High income: up to 6000 TL and having a flat . Middle income: 2500 TL and having a flat. Low income: 1000-1250 TL and tenant. Very low income: 600 TL and below. tenant.

When pregnant women were divided into two sub-groups according to their education status as high educated and low educated, all socio-demographic data were significantly different between sub-groups except pregnancy symptoms and the number of children (Table 4). But it should be noted that Mann-Whitney U analyses have limited statistical power to detect the differences of socio-demographic data between sub-groups due to small sample sizes. However, present study is not a socio-demographic study. Also, a clear difference in the socio-demographic status can be seen between sub-groups in the present study. High educated pregnant women (n=7) were all dentistry professionals, however, low educated pregnant women (n=7) were housewives (n=7), 4 for of them graduated from primary school; 3 of them from high school (Table 4).

In the sub-groups, when compared to pregnancy and lactation period. DMFT, SBI an PI indexes were found to be significantly increased and FR and CA activity to be significantly decreased in the lactation period in low-educated women (Table 5); Their salivary pH correlated positively with CA activity and P_i but negatively with DMFT in pregnancy period (Table 6). SBI index and Ca/Pi molar ratio were found to be significantly decreased and pH and BC to be significantly increased in lactation period in high educated women (Table 5). Salivary pH positively correlated with BC in both the 3rd trimester and lactation periods in this sub-group (Table 6).

Table 2. Dental, oral hygiene and salivary parameters in the main groups.

	Groups		
		Pregnant women	
	Non-pregnant Women (n=12) Mean±SD	Pregnancy period (n=14) Mean±SD	Lactation period (n=14) Mean±SD
DMFT	6.3 ± 3.6	6.6 ± 5	7.9 ± 6 [c]
SBI	0.3±0.6	0.4±0.3	0.72± 0.8 [c]
PI	0.62 ± 1	0.6 ± 0.6	1±1.2 [c]
Salivary Flow Rate			
(ml/min)	0.45±0.19	0.55±0.2	0.50±0.37
pH	7.1±0.5	6.3±0.5[b]	7.25±0.6[d]
Buffering Capacity	2.9±0.6	2.3±0.5[b]	2.9±0.7[c]
Free Calcium (mM)	0.53±0.22	0.75±0.22[a]	0.62±0.41
Phosphate (mM)	3.38±0.85	2.54±0.9[a]	3.0±1.7
Ca/P_i Molar Ratio	0.17±0.09	0.32±0.12[b]	0.24±017
Glutathione (µM)	10.4±2.8	8.3±4.4	9.4±5.5
Carbonic Anhydrase (kU/ml)	47±20	50±20	39±20
Protein (g/L)	1.94±1.09	1.95±0.81	1.80±0.79

Values are given as mean ± standard deviation (SD). t-test results: [a]p<0.05 and [b]p<0.01 significantly different from non-pregnant women; [c]p<0.05 and [d]p<0.01: significantly different from pregnancy period. DMF-T: decayed. missing and filled teeth. SBI: sulcus bleeding index. PI: plaque index

Table 3. Significant Pearson and Spearman correlation coefficients in non-pregnant control, 3rd trimester and lactation periods

	Groups		
		Pregnant women	
	Non-pregnant Women (n=12)	Pregnancy period (n=14)	Lactation period (n=14)
	Correlation coefficient (r)		
pH – Total protein	-	0.590*	-
pH – Carbonic anhydrase	-	0.520*	-
DMFT – SBI	-	0.620*	0.745**
SBI - PI	0.835**	0.802**	0.852**
Brush – Education‡	0.931**	0.992**	0.992**
Brush - Occupation	0.696*	0.937**	0.937**
Nutrition – SBI‡	0.650*	0.771**	0.829**
Nutrition – PI‡	0.650*	0.789**	0.914**
Education – SBI‡	0.868**	0.740**	0.821**
Education – PI‡	0.891**	0.878**	0.957**
Occupation – SBI‡	0.694*	0.693**	0.888**
Occupation – PI‡	0.771**	0.834**	0.904**

*p<0.05, **p<0.01, ‡Spearman correlations

Table 4. Characteristics of subgroups

	Pregnant Women		
	High Educated (n=7)	**Low Educated (n=7)**	***P value***
Age (mean±SD,Ye	31.3± 2.9	26.7±3.4	0.020*
Education [n(%)]			
Non-University	0 (0)	7 (100)	0.001#
PhD in Dentistry	7 (100)	0 (0)	
Occupation [n(%)]			
House wife	0 (0)	7 (100)	0.001#
Academician	7 (100)	0 (0)	
Socioeconomic status [n(%)]			
High	7 (100)	1 (14.3)	0.004#
Middle	0 (25)	1 (14.3)	
Low	0 (17)	2 (28.6)	
Very low	0 (0)	3 (43)	
Number of children [n(%)]			
Having no child	5 (71.4)	4 (57.1)	0.620#
Having one child	2 (28.6)	2 (28.6)	
Having 2 children	0 (0)	1 (14.3)	
Tootbrushing [n(%)]	7 (100)	0 (0)	
Twice a day	0 (0)	1 (14.2)	0.001#
Once a day	0 (0)	3 (42.9)	
Once a month Never	0 (0)	3 (42.9)	
Nutrition [n(%)]			
0	7 (100)	0 (50)	0.001#
1	0 (0)	5 (71.4)	
2	0 (0)	2 (28.6)	
Pregnancy Symptom			
Nausea	1 (14.3)	1(14.3)	1.0
Nausea and vomiting	1 (14.3)	1 (14.3)	
None	5 (71.4)	5 (71.4)	

Mann-Whitney U results except Age (*t-test results), **Nutrition;** 0: Regular diet with supported animal and vegetable foods, 1: Carbohydrate-based diet supported with animal and vegetable foods, 2: Carbohydrate-based diet, **Socioeconomic status;** High income: up to 6000 TL and having a flat , Middle income: 2500 TL and having a flat, Low income: 1000-1250 TL and tenant, Very low income: 600 TL and below, tenant.

Table 5. Dental, oral hygiene and salivary parameters in subgroups

	Pregnant Women			
	High Educated		**Low Educated**	
	Pregnancy period (n=7) Mean±SD	**Lactation period (n=7) Mean±SD**	**Pregnancy period (n=7) Mean±SD**	**Lactation period (n=7) Mean±SD**
DMF-T	3.6±3.6	3.6±3.6	9.7±4.5[ae]	12.14±4.2[cf]
SBI	0.18±0.13	0.05±0.12 [a]	0.6 ± 0.3[ae]	1.4 ± 0.5 [df]
PI	0.19±0.18	0.034±0.1	0.96 ± 0.6[bf]	2 ±0.8 [df]
Salivary Flow Rate (ml/min)	0.66±0.24	0.77 ± 0.37	0.44 ± 0.2	0.24± 0.06[cf]
pH	6.0±0.5	7.6 ± 0.4[b]	6.5 ± 0.5	6.9 ± 0.7
Buffering Capacity	2.1±0.4	2.9±0.8[a]	2.5±0.58	2.9±0.6
Free Calcium (mM)	0.72±0.28	0.47±0.28	0.77±0.15	0.77±0.48
Phosphate (mM)	2.5±0.9	3.7±2.1	2.6±0.92	2.4±0.98
Ca/P_i Molar Ratio	0.30±0.13	0.13± 0.04[a]	0.33± 0.11	0.35± 0.19[e]
Glutathione (μM)	9.8±5.2	9.6±7.7	6.7±2.8	9.3±3.0
Carbonic Anhydrase (kU/ml)	46±20	54±20	51±20	23±10[df]
Protein (g/L)	1.69±0.73	1.87 ± 0.99	2.22±085	1.73±0.61

Values are given as mean ± SD. t-test results: [a]p<0.05 and [b]p<0.01 significantly different from high educated pregnancy period, [c]p<0.05 and [d]p<0.01 significantly different from low educated pregnancy period, [e]p<0,05 and [f]p<0,01 significantly different from high educated lactation period. DMF-T: decayed, missing and filled teeth, SBI: sulcus bleeding index, PI: plaque index. DMF-T: decayed, missing and filled teeth, SBI: sulcus bleeding index, PI: plaque index.

Table 6. Significant Pearson correlation coefficients in subgroups

	High educated		**Low educated**	
	Pregnancy period (n=7)	**Lactation period (n=7)**	**Pregnancy period (n=7)**	**Lactation period (n=7)**
	Correlation coefficient (r)			
pH – Buffering capacity	0.926**	0.801*	-	-
pH – Carbonic Anhydrase	-	-	0.781*	-
pH – Phosphate	-	-	0.813*	-
pH – DMFT	-	-	-0.802*	-

Discussion

DMF-T, SBI and PI

The caries tendency may increase with the increase of gingivitis intensity in pregnancy periods (3, 4, 26). Although pregnancy gingivitis has been recognized as a common disease for many years (27), only recently studies on caries tendency during gestation have been conducted, (3, 4, 26). However, no detailed study of caries status during pregnancy and lactation periods have been examined. In the present study, the increase in DMFT was found from the 3rd trimester of pregnancy to early lactation. The physiological conditions of pregnancy may have both an initiating and accelerating effect on the precavitated lesions from the beginning of pregnancy to 6-8 week lactation period. We found significant positive correlation between DMFT and SBI indexes in pregnant women in consistent with previous studies (3, 26). We also found the same relationship in lactating women. Pregnancy gingivitis is characteristically self-limiting and it abates in postpartum with the decline in hormone production (28) and gingival inflammation resolves when pregnant women maintain correct oral hygiene procedures during their gestation (29). However, in the present study, SBI and PI levels increased from pregnancy to lactation period. A strong positive correlation was also found between these parameters in both pregnant and lactating groups. This association is consistent with the other reports that had attributed such conditions to bad oral hygiene in pregnancy (30). In contrast to our results, recent longitudinal studies have reported a decrease or no change in oral hygiene parameters in the women with good oral hygiene between the 3rd trimester of pregnancy and lactation periods (29, 31),

In the present study, half of the pregnant women were dentists with PhD degree (high educated sub-group) and the other half were lower educated. We found the increase in caries tendency in the low educated sub-group. Caries may also progress slowly or rapidly, the rate depending upon many factors such as diet, saliva composition. the number of bacteria, oral hygiene and other habits (32). It is widely believed that the development of caries usually takes several

years in adults, however, sometimes it may develop as quickly as seen in primary dentition (33). It has been reported that the numbers of teeth requiring restoration are correlated negatively with educational level and with the perception of dental health in pregnant women (34). The present study found that the low educated pregnant women had significantly higher DMFT indices than the professionals. On the other hand, they had the teeth needing restoration, however, there were none in professionals. Cariogenic bacteria counts have been reported to be correlated with the number of decayed tooth surfaces in pregnant women (6). Although we did not perform cariogenic bacteria counts, it is likely that the higher numbers of unrestored teeth in the low educated group would have led to higher bacteria levels, which in turn would have led to higher DMFT in the present study. So, we may suggest that caries tendency in our study seems to be more attributable to poor oral hygiene habits rather than only direct consequence of pregnancy as such.

It has been reported that low socio-demographic background also negatively influences plaque accumulation and gingival health during pregnancy (35, 36), especially in low-educated pregnant women (3). In consistency with these results, despite small sample sizes of our sub-groups, SBI and PI were increased in our low educated pregnant sub-group from the 3rd trimester of pregnancy to lactation period. It should be emphasized and mentioned that our pregnant participants did not take any professional dental intervention during their gestation and 6-8 week early lactation, but they were instructed about personal oral hygiene care during their pregnancy by an experienced dentist. However, they failed four of oral hygiene possibly resulting from the fact that they were poor or had lower awareness of the importance of oral hygiene during pregnancy. However, in professionals, there were no alteration in PI but SBI was decreased significantly from the 3rd trimester of pregnancy to lactation in the present study. In professionals, the decrease in SBI is definitely related with correct oral hygiene procedure (31). It has been reported that the periodontal state of women with higher education and the intellectuals was much better than that of the less educated patients (26). Present results support these findings since SBI and PI were significantly lower in the women with good oral perception than the women who neglect oral hygiene.

pH, BC and FR

Salivary flow rate (FR) is the most important parameter since the cariostatic activity or efficacy of certain salivary parameters depend upon it (1). Decreases in saliva secretion are responsible for increasing plaque formation and creating a risk for dental caries (37). Salivary pH and BC are among the main factors affecting the stability of enamel (38). These factors modified by pregnancy, such as increasing acidity, reducing BC and promoting bacterial growth play a pivotal role in cariogenesis (7, 9).

The significantly lower levels of salivary pH and BC levels in pregnancy group than in non pregnant-control and lactation groups; and no differences in FR between groups are consistent with reports in previous cross-sectional and longitudinal salivary studies (7-9). The positive correlation was found between pH and TP in pregnant group in the present study. The decrease in oral pH in 3rd trimester of pregnancy may be related with the decrease in salivary protein contents.

In the low educated sub-group, when lactation period compared with its pregnancy period, the significant decrease in FR was consistent with significant increase in DMFT. The increase in DMFT usually accompanies a decrease in FR in healthy subjects (39). Dietary factors may have been negatively affected on FR in this group (40). Their pH and BC were not changed significantly between periods.

In the high educated group, while pH and BC were reached normal levels from pregnancy to lactation period, FR was not changed significantly. Additionally, a significant correlation between pH and BC was seen in both 3rd trimester of pregnancy and lactation periods in this group. Therefore, we may also speculate that the positive correlation between pH and BC in high educated group suggests that all salivary buffering parameters work properly in the regulation of oral pH in both 3rd trimester of pregnancy and lactation periods and thus, salivary pH reached normal levels in the lactation.

GSH

There is no study about salivary GSH in pregnancy in the literature. However it has been reported that plasma GSH levels of pregnant women were not significantly different from those of non-pregnant women (41) due to the development of possible adaptive mechanisms against the inflammation during healthy pregnancy (42). Moreover, the significant increase in plasma GSH level has been reported in lactation period than those in 3rd trimester of pregnancy (43) and again it has been suggested that the development of adaptive mechanism to protect the baby from environmental toxicity may be involved in this increase of GSH (44).

The present study is the first report on salivary GSH and pregnancy and lactation periods. No significant differences found in salivary GSH levels between non-pregnant, pregnant and lactating women are compatible with plasma GSH levels in pregnancy written above.

In our previous study we found that salivary GSH level was lower in young subjects with caries than those without caries and it was correlated with DMF-T (14). Similarly salivary GSH was negatively correlated with DMF-T in pregnancy period in the present study.

In the present study, the reason why we did not find a significant increase in GSH levels in lactation period than those in pregnancy, may be due to the increase in caries activity and oral hygiene in lactation than pregnancy and non-pregnant condition and may be due to the lactation physiology.

In low educated sub-group, there was non-significant decrease in salivary GSH level of 3rd trimester of pregnancy compared to lactation period. However caries activity and oral hygiene parameters had been increased significantly towards the lactation period from 3rd trimester of pregnancy. The decrease in salivary GSH may indicate that caries process becomes activated during the 3rd trimester of pregnancy, and lactation physiology was more effective than caries activity in lactation period.

In high educated sub-group, no significant difference in GSH levels between 3rd trimester of pregnancy and lactation period may be related to pregnancy and lactation physiology or it may be related to unchanged dental and periodontal parameters.

CA

Salivary carbonic anhydrase (CA VI, EC.4.2.1.1, a zinc metalloenzyme) is the only enzyme that catalyzes the salivary bicarbonate buffer system (the main buffer system of saliva). Low CAVI concentrations in the saliva were associated with the

prevalence of dental caries, especially in individuals with poor oral hygiene (14).

In the present study, salivary CA activity did not differ significantly between non-pregnant controls, pregnancy and lactation groups. This finding was consistent with the results of the study on non-pregnant, pregnant and lactating women with good oral health and a history of regular dental appointments (8). However, the positive correlation that we found between CA activity and pH in the 3rd trimester of pregnancy was not reported in the previous study (8).

In the present study, salivary CA activity was significantly different between sub-groups. It decreased significantly in lactation period compared to its pregnancy period in low educated sub-group and compared to high educated lactation period. This decrease was parallel with the decrease of FR in low educated lactation group compared to its pregnancy period. It has been reported that FR was positively correlated with bicarbonate concentration in unstimulated saliva in healthy individuals (45, 46). However, the recent pregnancy study has reported that only stimulated salivary CA is slightly influenced by FR with a weak positive correlation in late pregnancy but not in lactation. This relationship has been suggested as a non-specific one in that study (8). In consistent with this suggestion, there were no correlations between CA and FR in both periods in low educated group in the present study. Only their parallel decreases were present. The decrease in CA in the lactation period may be due to bad oral hygiene rather than the decrease in salivary FR in the low educated sub-group. It was consistent with earlier report on healthy person with bad oral hygiene (47).

In low educated sub-group, salivary pH was correlated positively with CA in the 3rd trimester of pregnancy, however neither pH and nor BC levels changed significantly from 3rd trimester to lactation period. Contrary to earlier suggestions in a study in pregnant women (8), CA seems to be directly involved in the regulation of actual salivary pH in pregnant women with high DMFT level as seen in the present study.

Ca and P

The role of salivary free Ca and P_i ions in demineralization and remineralization processes has been clearly established (1). A recent in vitro study has suggested that remineralisation occurs at low Ca/P_i molar ratio and high P_i levels within the simulated saliva formulations (48). It has been reported that salivary total Ca and P levels were found to be decreased (49) and salivary free Ca levels to be increased (50, 51) and P_i to be decreased (52, 53) in healthy subjects with caries . Although salivary total Ca levels have been considerably investigated (7, 9) and are generally reported to be lower during pregnancy than in non-pregnant controls in both stimulated and unstimulated whole saliva (7). free Ca level in saliva has not been studied in pregnancy yet. The present study is the first one related salivary free Ca in pregnancy and lactation period. We found that the pregnant group salivary free Ca levels were to be significantly higher and P_i levels were to be lower than in non-pregnant controls. Our findings related with P_i was consistent with the findings of two studies (54, 55) but not another study of pregnancy (9).The present Ca and P_i results. higher Ca/P_i molar ratio, lower pH and BC indicate demineralization in pregnancy group. Non-significant increase in DMF-T values in lactation group compared to pregnancy, may also support demineralization. It has been reported that salivary free Ca level in lactation decreased in lactation period as a concequence of production of maternal milk (56). In lactation period of the present study, salivary free Ca level which is suggested to increase with the caries activity may be overshadowed by the decreasing effect of lactation physiology.

Since DMF-T increased in lactation period compared to pregnancy, demineralization was also evident in low educated sub-group. However, salivary Ca and Pi levels did not significantly change between periods in this sub-group. In addition, both negative correlation between pH and DMF-T, and positive correlation between pH and P_i support demineralization in this group. On the other hand, the lower FR and CA levels may explain why we found new decay in lactation period of low educated subgroup.

In high educated group, Ca and P_i levels did not also significantly change between pregnancy and lactation periods. However, the increased Ca/P_i ratio and decreased pH and BC in pregnancy period compared to lactation period may suggest the occurrence of demineralization in pregnancy and remineralization in lactation. Additionally, a significant correlation between pH and BC was seen in both 3rd trimester of pregnancy and lactation periods in this group.

Within the limitation of our subgroups sample size, although their underlying mechanisms of action are not fully understood, daily oral hygiene care and good nutrition seem to have an effective impact on maintaining oral and salivary parameters in pregnancy and maintaining and/or improving them between the 3rd trimester and lactation period.

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