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Original Article

Dentistry Section

Estimation of DMFT, Salivary Streptococcus Mutans Count, Flow Rate, Ph, and Salivary Total Calcium Content in Pregnant and Non-Pregnant Women: A Prospective Study

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ABSTRACT

Introduction: Pregnancy, a period from conception till birth, causes changes in the functioning of the human body as a whole and specifically in the oral cavity that may favour the emergence of dental caries. Many studies have shown pregnant women at increased risk for dental caries, however, specific salivary caries risk factors and the particular period of pregnancy at heightened risk for dental caries are yet to be explored and give a scope of further research in this area.

Aim: The aim of the present study was to assess the severity of dental caries in pregnant women compared to non-pregnant women by evaluating parameters like Decayed, Missing, Filled Teeth (DMFT) index, salivary *Streptococcus mutans* count, flow rate, pH and total calcium content.

Materials and Methods: A total of 50 first time pregnant women in the first trimester were followed during their second trimester, third trimester and postpartum period for the evaluation of DMFT by World Health Organization (WHO) scoring criteria, salivary flow rate by drooling method, salivary pH by pH meter, salivary total calcium content by bioassay test kit and salivary *Streptococcus mutans* count by semiautomatic counting of colonies grown on Mitis Salivarius (MS) agar supplemented by 0.2U/ml of bacitracin and 10% sucrose. The observations of pregnant women were then compared with same parameters evaluated in the 50 non-pregnant women. Paired t-test and Wilcoxon sign rank test were performed to assess the association between the study parameters.

Results: Evaluation of different caries risk factors between pregnant and non-pregnant women clearly showed that pregnant women were at a higher risk for dental caries. Comparison of caries risk parameters during the three trimesters and postpartum period showed that the salivary *Streptococcus mutans* count had significantly increased in the second trimester, third trimester and postpartum period while the mean pH and mean salivary total calcium content decreased in the third trimester and postpartum period. These changes reflected on the DMFT score which increased in the third trimester and postpartum period.

Conclusion: The results of this study suggest that there is a definite correlation between pregnancy and dental caries. We conclude that the third trimester and postpartum period of pregnancy are the periods during which the pregnant women are at a higher risk for development of dental caries.

Keywords: Dental caries, Mitis salivarius agar, Pregnancy

INTRODUCTION

"Every child costs the mother one tooth"

--Anonymous

This ancient dictum means that with every childbirth, the mother loses one tooth. In the literature, it is recorded that women who gave birth to more children show a higher percentage of 'decays' compared to women with only one child [1].

The development of dental caries occurs through a complex process carried out by cariogenic microorganisms colonizing on the tooth surface. Marked reduction in pH in the presence of a sugar substrate are the requisites to induce dental caries [2]. *Streptococcus mutans* has been proved to be an efficient cariogenic microorganism in many studies. The risk of dental caries increases in the presence of the microorganism if host's defense mechanisms do not override the bacteria [3]. Pregnant women are generally considered at an increased risk for dental caries probably due to the intraoral changes associated with hormonal changes and the delay in the treatment [4].

Pregnancy is a systemic condition that shows changes in the whole body but more specifically in the mouth making it a favourable environment for the emergence of dental caries. Many studies have shown pregnant women at increased risk for dental caries [5], however what specific salivary caries risk factors are involved and which particular period of pregnancy shows a heightened risk for dental caries is yet to be explored and gives a scope of further research in this area.

The present study was conducted to assess and correlate the severity of dental caries in pregnant women compared to non-pregnant women by evaluating different parameters like salivary *Streptococcus mutans* count, DMFT index, salivary flow rate (SFR), salivary pH and total salivary calcium content. The objectives of the study were to find out: (i) the specific salivary dental caries risk factor(s) involved in the causation of dental caries in pregnant women; and (ii) the particular period during which the pregnant women showed the highest risk for dental caries.

MATERIALS AND METHODS

The present prospective study was conducted at the Krishna Institute of Medical Sciences Deemed University (KIMSDU), Karad, Maharashtra, India. The monthly prevalence of first time pregnant women of the first trimester at KIMSDU hospital was estimated

to be an average of 3%. Based on this data the sample size was estimated to be 46.56 but for rounding off, 50 patients were included in the study groups.

Fifty first time pregnant women in the first trimester (later followed in the second trimester, third trimester and postpartum period) with age ranging between 18 to 28 years consulting Department of Gynaecology and Obstetrics were considered as a study group. Fifty non-pregnant women of same age group were considered as a control group.

The pregnant women with a history of any systemic diseases, or on antimicrobial therapy since two months or during the study, or using any form of medication modifying saliva, or showing clinical signs of xerostomia were excluded from the study.

The research protocol was submitted to the Institutional Ethical Committee and ethical committee clearance was obtained before commencing the study. Informed consent was obtained from the study participants. The study was conducted between January 2014 to June 2015.

A DMFT score was used to express the caries experience. A high DMFT score (15) indicated an active caries process before pregnancy. The pregnant women with DMFT index ≥ 15 were considered as already a high-risk group and were excluded from the study. The pregnant women were evaluated in the following schedule: sixth week of pregnancy (first trimester); 18th week of pregnancy (second trimester); 30th week of pregnancy (third trimester) and sixth week after delivery (Postpartum period).

All the clinical procedures were carried out in the late morning (around 9 am to 11 am), in a comfortable room free of noise and distraction. At the start of the examination, a thorough case history was taken to record the details of name, age, address, phone number, family history, personal history, medical history and dental history.

The intraoral examination was started with the recording of DMFT indices in accordance with WHO criteria [6]. Following the DMFT scoring, the saliva collection was carried out. The subjects were seated comfortably on the chair. The collection of unstimulated whole saliva was performed under resting conditions. Following standard procedures, subjects were asked to wash their mouth and sit passively for 10 min as the saliva accumulated on the floor of the mouth and expectorate in a relaxed position with their heads bent forward, allowing the saliva to drool through the open lips into a sterile graduated collection cup.

Sialometric Analysis

Immediately after collection, the saliva volume was measured and then the salivary flow rate was calculated in ml/10 minutes. The collected samples were maintained in ice after collection and transported to the microbiology department, not exceeding half hour period since beginning of the sample collection.

Sialochemical Analysis

Total calcium content of saliva was measured using BIOASSAY system kits (Calcium test kits) [7] and salivary pH was measured electrometrically using the pH meter.

Microbiological Assay [Table/Fig-1]

Mitis Salivarius (MS) agar was one of the first media to be developed as a selective medium for culturing *streptococci* species in general. The addition of 0.2U/ml of bacitracin and 10% sucrose has led to an improved medium Mitis Salivarius Bacitracin (MSB) with a high selection of *Streptococcus mutans* [8]. Aliquots of 0.5 ml of saliva were diluted in a 10 fold solution of sterile Phosphate buffered saline (0.05M; pH 7.3) and 20 microliters (µl) was plated on Mitis-Salivarius agar supplemented with bacitracin (0.2 units/ml) and 10% sucrose. The plates were incubated in a 5% carbon dioxide (CO_2) environment at 37^{0}C for 48 hours.

[Table/Fig-1]: Collection of saliva, plating of aliquots of saliva on MSB agar, incubation in 5% CO_2 environment, *Streptococcus mutans* colonies, and semiautomatic counting of colonies.

Following incubation, the colonies were identified by morphological characteristics of the bacteria. The *Streptococcus mutans* can be distinguished from other species by raised, convex, undulate, opaque, pale blue colonies giving a granular "frosted glass" appearance [8]. The identification of *Streptococcus mutans* was confirmed by gram staining and biochemical tests like catalase, mannitol and sorbitol fermentation of randomly selected samples.

The colony counting of Streptococcus mutans was done under a colony counter and the number of 'Colony Forming Units (CFU)' were multiplied by the number of times the original ml of sample was diluted and expressed as the number of colony forming units per milliliter (CFU/ml).

The colonies were scored following Berkowitz et al., criteria [9].

0 = no growth

1 = 1-10^3

2 = 10^3-10^5

3 ≥ 10^5 CFU per ml of saliva.

STATISTICAL ANALYSIS

Statistical package for the social science software (SPSS Inc. released 2007. SPSS for Windows, version 16.0. Chicago) was used for the statistical analysis of the collected data. Data was scrutinized using the descriptive statistics and comparison of different variables between the study and control group. The associations between the study parameters were assessed by performing the paired t-test and Wilcoxon sign rank test. The p-value of ≤ 0.05 was considered significant.

RESULTS

Total 50 pregnant and 50 non-pregnant women completed the study. The mean age of the pregnant women in the study was 23.92 years and for the control group was 22.16 years.

The scoring of DMFT index was carried out in the three trimesters of pregnancy and in the postpartum period which was then compared with the DMFT scores of the control group. By using Wilcoxon sign rank test (p-value < 0.05) there was a significant difference between the median DMFT at third trimester and the postpartum period when compared to the control group [Table/Fig-2].

The mean CFU/ml had significantly increased during the second, third trimester and postpartum period of pregnancy when compared to the control group (p<0.01). The CFU/ml in the first trimester, however, did not show any significant difference compared to the control group [Table/Fig-3].

The comparison of salivary flow rates between the pregnant and non-pregnant women showed that the flow rate had statistically increased during the second trimester of pregnancy (p<0.05), while no difference was noted in the first, third trimesters and the postpartum periods [Table/Fig-4].

There was decrease in the salivary pH particularly during the third trimester and postpartum period compared to control group with a p-value less than 0.01. There was no significant difference in salivary pH in first and second trimester compared to the salivary pH of the control group [Table/Fig-5].

A significant decrease in the salivary total calcium content was noted in the third trimester and postpartum period ($p<0.01$) while the first and second trimester did not show any statistical difference when compared to the control group [Table/Fig-6].

DISCUSSION

Dental caries can be defined as a diet and saliva modified bacterial disease [6]. Pregnancy represents a particular physiological state, characterized by hormonal and metabolic changes with transient nature, which can influence the oro-dental status with repercussions in terms of increasing the number of caries and their accelerated evolution [1]. Salivary analysis has become an important resource for the evaluation of salivary conditions with physiologic and pathologic implications and is a useful tool for disease diagnosis, mainly due to its origin, composition, functions and interactions with other organ systems [10].

Dental caries develops over a considerable period of time and shows the fluctuation of the bacterial counts in response to the changing oral environment. It is well known that a single saliva sample records the microbial counts at a particular point of time [11]. Therefore, in this cross-sectional study, the *Streptococcus mutans* count over the three trimesters of pregnancy and postpartum period was evaluated. All the pregnant women in the present study were infected by *Streptococcus mutans* similar to the study conducted by Herrera GCL et al., who showed that all the pregnant women (100%) of the study group were infected by the microbe [12].

The studies undertaken previously to estimate the stimulated and unstimulated SFR between pregnant and non-pregnant women have shown mixed results. Our finding showed an increase in SFR in the second trimester of pregnancy while the study conducted by Naveen S et al., in 2013 found a significant increase in the SFR in the second trimester pregnant women compared to non-pregnant women [10]. Our findings are contrary to the study conducted by Abrao ALP, et al., in 2014 who concluded that the median unstimulated SFR showed no differences among trimesters of pregnancy [13]. Pregnancy induces decreased gastroesophageal sphincter tone and prolonged gastric emptying times. These changes along with decreased esophageal tone lead to ptyalism. Further, decreased large bowel motility leads to increased water absorption and constipation. Hence these factors have been hypothesized to the increase in the saliva flow [10].

Salivary pH is one of the main factors playing a pivotal role in cariogenesis by affecting the stability of enamel. It gets modified during the pregnancy causing an increased acidity that promotes the bacterial growth [6]. Our finding of decreased salivary pH in third trimester of pregnancy are similar to the finding of Oztruk L

Period	DMFT score							Median	Total
	0	1	2	3	4	5	6		
1st trimester	6	4	17	11	7	3	2	2	50
2nd trimester	6	4	17	11	7	3	2	2	50
3rd trimester	6	4	14	13	8	3	2	3*	50
Post partum	6	4	14	13	8	3	2	3*	50
Control	2	5	21	15	4	1	2	2	50

[Table/Fig-2]: Comparison of DMFT score in pregnancy, postpartum period and the control group.
*Statistically significant, p-value <0.05.
By using Wilcoxon sign rank test (p-value < 0.05) there is a significant difference between median DMFT at third trimester and the postpartum period compared to the control group.

CFU at	Number of cases	*Streptococcus mutans* CFU ($x10^5$ /ml)					p-value
		Min	Max	Mean	SD	Median	
1st trimester	50	1.37	5.30	3.21	0.82	2.84	0.463
2nd trimester	50	1.30	5.75	3.71	1.03	3.77	0.001*
3rd trimester	50	2.37	5.79	4.61	0.88	4.75	0.001*
Post partum	50	1.70	5.82	4.50	1.09	4.74	0.001*
Control	50	1.26	4.79	3.33	0.81	3.45	----

[Table/Fig-3]: Comparison of *Streptococcus mutans* CFU/ml in three trimesters of pregnancy and the postpartum period against the control group.
* Statistically significant, p-value <0.05.
CFU = Colony Forming Units
By using paired t-test (p-value < 0.05) there is a significant difference between mean CFU/ml at second trimester, third trimester and postpartum period compared to the control group.

SFR at	Number of cases	SALIVARY FLOW RATE (SFR) in ml/10 min					p-value
		Min	Max	Mean	SD	Median	
1st trimester	50	6	11.30	8.51	1.18	8.65	0.400
2nd trimester	50	7	12.00	9.09	1.24	9.00	0.020 *
3rd trimester	50	7	12.00	9.01	1.36	9.00	0.093
Post partum	50	6	11.30	8.23	1.22	8.00	0.252
Control	50	6	11	8.31	1.19	8	----

[Table/Fig-4]: Comparison of SFR in three trimesters of pregnancy and the postpartum period against the control group.
* Statistically significant, p-value <0.05.
By using paired t-test (p-value <0.05) there is a significant difference between mean SFR between second trimester and the control group.

pH at	Number of cases	pH					p-value
		Min	Max	Mean	SD	Median	
1st trimester	50	6.8	7.70	7.23	0.20	7.25	0.833
2nd trimester	50	6.8	7.70	7.20	0.19	7.20	0.337
3rd trimester	50	6.4	7.70	6.95	0.24	7.00	0.001*
Post partum	50	6.4	7.40	6.92	0.22	7.00	0.001*
Control	50	6.4	7.8	7.24	0.27	7.2	---

[Table/Fig-5]: Comparison of salivary pH in three trimesters of pregnancy and the postpartum period against the control group.
* Statistically significant, p-value <0.05.
By using paired t-test (p-value < 0.05) there is a significant decrease in the mean pH at third trimester and postpartum period when compared to the control group.

Calcium content at	Number of cases	Salivary total calcium content in mmol/dl					p-value
		Min	Max	Mean	SD	Median	
1st trimester	50	4.2	5.90	5.30	0.43	5.35	0.999
2nd trimester	50	4.1	5.90	5.39	0.46	5.40	0.200
3rd trimester	50	4	5.80	4.91	0.46	4.90	0.001*
Post partum	50	4.1	5.90	4.82	0.43	4.85	0.001*
Control	50	4.2	5.9	5.30	0.43	5.35	----

[Table/Fig-6]: Comparison of salivary total Calcium content in three trimesters of pregnancy, postpartum period against the control group.
* Statistically significant, p-value <0.05.
mmol/dl = millimole per decilitre.
By using paired t-test (p-value < 0.05) there is a significant decrease in the mean total salivary calcium content at third trimester and postpartum period compared to the control group.

Period Parameters	1st trimester	2nd trimester	3rd trimester	Postpartum period
CFU/mL	-----	↑	↑	↑
SFR	-----	↑	-----	----
pH	-----	-----	↓	↓
Ca	-----	-----	↓	↓
DMFT	------	-----	↑	↑

[Table/Fig-7]: Summary of correlation of different parameters in pregnant women compared to non-pregnant women.
↑ = statistically increased
↓ = statistically decreased
CFU = *Streptococcus mutans* Colony forming units per ml
SFR = Salivary flow rate
pH = Salivary pH
Ca = Salivary total calcium content
DMFT = Decayed, Missing and Filled teeth

et al., and Naveen S et al., [6,10], however in our study there was decrease in salivary pH in postpartum period which is in contrast to the findings of Oztruk L et al., who found no significant difference in salivary pH in pregnant women during lactation period compared to the control group [6]. Jain K and Kaur H reported in their study a progressive decrease in salivary pH from first trimester to third trimester of pregnancy consistent with our findings of a continuous drop in pH particularly during third trimester and postpartum period [14].

The reduction in pH value during pregnancy is related to the effect of progesterone hormone, which is known to decrease plasma bicarbonate level during pregnancy resulting in a decrease in the pH and buffering capacity [10].

Calcium is an important component of saliva and any change can affect teeth and oral health [7]. Salivary total calcium levels are generally reported to be lower during pregnancy than in non-pregnant controls in both stimulated and unstimulated whole saliva [6].

Many studies such as those conducted by Salvolini E et al., Guidozzi F et al., and Rockenbach MI et al., on the evaluation of the total calcium content of saliva reported no significant difference in pregnant women compared to the non-pregnant women [15-17]. Our finding is similar to the study conducted by Oztruck L et al., who showed that there was significantly decreased salivary calcium activity in postpartum period compared to pregnancy [6]. Rio R et al., found a significant decrease in salivary calcium in the first trimester of pregnancy in contrast to our study where the significant decrease was noted during third trimester and postpartum period while first and second trimester did not show any significant decrease in total calcium content of saliva [18].

Dental caries is the second most important disease of the oral cavity in pregnancy. The DMFT index indicates the status of the oral cavity with regard to the number of teeth with decay, fillings and extracted for caries [19]. In our study, we found that there was a significant difference between median DMFT at third trimester and postpartum period compared to the control group. Our findings are consistent with Ozturk L et al., who found a significant increase in DMFT from third trimester of pregnancy to early lactation [6]. Bressane L et al., found 100% caries prevalence in their study population of 50 pregnant women showing a high average DMFT score of 10 and average decayed teeth (D) score of 2.52 [5]. In our study, the caries prevalence was 88%. The DMFT score ranged from 0 to 6 and the average DMFT index score was two for first and second trimester and three for third trimester and postpartum period. The increase in DMFT index in the third trimester and postpartum period seen in our study can be due to the physiological conditions of pregnancy that show both an initiating and accelerating effect on the precavitated lesions from the beginning of pregnancy to 6-8 weeks lactation period [6].

Most studies regarding SFR have reported no significant changes in pregnant and non-pregnant women. In addition, the findings regarding SFR throughout pregnancy are controversial, suggesting that many factors may regulate the flow during pregnancy [18]. From our study, it can be concluded that the total salivary *Streptococcus mutans* count in pregnancy shows no significant relation with the SFR.

The salivary pH in our study showed a significant decrease during the third trimester and postpartum period and the salivary *Streptococcus mutans* count showed a consistent increase in the third trimester and postpartum period. Hence in our study, there was a significant increase in salivary *Streptococcus mutans* counts as the pH decreased.

The reduction in pH value during pregnancy is related to the effect of progesterone hormone, which is known to decrease plasma bicarbonate level during pregnancy resulting in a decrease in the pH and buffering capacity [10]. Salivary pH and buffering capacity are among the main factors affecting the stability of enamel. These factors modified by pregnancy, such as increasing acidity, reducing the buffering capacity and promoting bacterial growth play a pivotal role in cariogenesis [6]. Cariogenic bacteria such as *Streptococcus mutans* survive and grow in the mouths of caries active patients due to the low resting pH in the plaque environment [13]. This could explain the reason for the particular increase in the *Streptococcus mutans* colonies in late pregnancy and the postpartum period when the salivary pH becomes more acidic.

There was a significant increase in DMFT index in the third trimester and postpartum period with an increase in salivary *Streptococcus mutans* count. This positive correlation has been reported by many studies. Studies have shown that *Streptococcus mutans* is an efficient cariogenic microorganism. *Streptococcus mutans* colonizes tooth surface causing marked reduction in pH in presence of sugar substrate and consequently cause dental caries [2].

In our study, there was a significant decrease in the total calcium content of saliva and a significant increase in salivary *Streptococcus mutans* count in the third trimester of pregnancy and the postpartum period. Hence, as the calcium content of the saliva decreased, the *Streptococcus mutans* colony forming units increased.

Calcium is an important component of saliva and any change can affect teeth and oral health. Enamel is mainly formed by hydroxyapatite (calcium and phosphorous); saliva causes enamel tooth maturation after the eruption of teeth as well as protecting it in the oral cavity. Calcium ion in saliva helps to balance hard dental tissue and therefore reduction in its concentration during pregnancy may increase caries [7].

No definite correlation was seen between the SFR and the DMFT index. The SFR had increased during the second trimester while DMFT index had increased in third trimester and postpartum period. From the present study it can be concluded that the SFR does not show any effect on the progression of the dental caries.

A statistically significant decrease in DMFT score and salivary pH was noted in third trimester and postpartum period. The decrease in salivary pH contributes to acidic conditions leading to dental caries development [18].

A definite correlation could be found between the DMFT score and total salivary calcium content during third trimester and postpartum period. From this study it can be concluded that low salivary calcium content are associated with dental caries.

The findings of the present study deduced after carrying out the microbiological and salivary analysis are summarized in the tabular format [Table/Fig-7]. In summary, the pregnant women showed decrease in salivary flow rate, decrease in total salivary calcium content but no relevant changes in SFR in their third trimester and postpartum period. These altered oral conditions presented favourable environment for colonization of *Streptococcus mutans* and concomitant development of dental caries.

LIMITATION

The limitation of the present study is that all the salivary parameters were not included and counting of colonies was semi automatic rather than fully automatic. Also, the sample size was limited to generalize the findings to a wider population.

CLINICAL IMPLICATIONS AND FUTURE PROSPECTS

The present study clearly shows that pregnant women are at a high risk of dental caries particularly during their third trimester and postpartum period. Also, many pregnant women particularly the first time pregnant women do not visit the dental clinics for treatment or instructions regarding the oral health care [20]. Therefore, there are increased chances of vertical transmission of *Streptococcus mutans* to their children. This study suggests urgent, compulsory and early oral health care education and dental services particularly for the

first time pregnant women in their first trimester to avoid any dental pain and infection in the following trimesters and in the postpartum period during which the dental treatment can be tedious.

CONCLUSION

The findings of our study revealed that there is a definite correlation between pregnancy and dental caries. Within the limits of this study, we can thus conclude that the third trimester and postpartum period of pregnancy are the periods during which the pregnant women are at a higher risk for development of dental caries.

Conducting more of such longitudinal studies would be valuable to establish the temporal association between pregnancy and dental caries. Therefore, future research should continue to evaluate the specific association between the particular period of pregnancy and development of dental caries. This knowledge may form the basis for targeting preventive and therapeutic measures for pregnant women who are at greatest risk for dental caries. Physicians should understand the potential relationship between pregnancy and dental caries, so as to educate patients about their oral disease risk factors and reinforce the benefits of healthy lifestyle.

REFERENCES

[1] Molnar-Varlam C, Molnar-Varlam C, Gabriela B, Tohati A. Risk assessment of caries in pregnancy. Acta Medica Marisiensis. 2011;57(6):685-89.
[2] Ravindran S, Chaudhary M, Gawande M. Enumeration of salivary *Streptococci* and *Lactobacilli* in children with differing caries experience in a rural Indian population. ISRN Plastic Surgery. 2013;2013:01-06.
[3] Ferraro M, Vieira AR. Explaining gender differences in caries: (a multifactorial approach to a multifactorial disease. Int J Dent. 2010;2010:649643.
[4] Umoh A, Azodo C. Nigerian dentists and oral health care of pregnant women: Knowledge, Attitude and Belief. Sahel Med J. 2013;16(3):111-15.
[5] Bressane L, Costa L, Vieira J, Rebelo M. Oral health conditions among pregnant women attended to at a health care centre in Manaus, Amazonas, Brazil. Rev Odonto Cienc. 2011;26(4):291-96.
[6] Oztruk L, Akyuz S, Garan A, Yarat A. Salivary and dental - oral hygiene parameters in 3rd trimester of pregnancy and early lactation: the effect of education. Marmara Dental Journal. 2013;1:01-08.
[7] Bakhshi M, Sabet MS, Hashemi ES, Bakhtiari S, Tofangchiha M, Marhabi SA, et al. Evaluation of biochemical changes in unstimulated salivary, calcium, phosphorous and total protein during pregnancy. Afr J Biotechnol. 2012;11(8):2078-83.
[8] Wan AKL, Seow WK, Walsh LJ, Bird PS. Comparison of five selective media for the growth and enumeration of *Streptococcus mutans*. Aust Dent J. 2002;47(1):21-26.
[9] Berkowitz RJ, Turner J, Green P. Primary oral infection of infants with *Streptococcus mutans*. Arch Oral Biol. 1980;25:221e4.
[10] Naveen S, Asha ML, Shubha G, Bajoria AA, Jose AA. Salivary Flow Rate, pH and buffering capacity in pregnant and non-pregnant women - a comparative study. JMED Research. 2014;2014:01-08.
[11] Pannu P, Gambhir R, Sujlana A. Correlation between the salivary *Streptococcus mutans* levels and dental caries experience in adult population of Chandigarh, India. Eur J Dent. 2013;7:191-95.
[12] Herrera GCL, Pantoja FP, La Maza T, Sanhueza CA, Salazar NLA. Microbiologic and molecular diagnostic of cariogenic bacteria in pregnant women from the Araucania Region of Chile. Rev Chilena Infectol. 2008;4:270-75.
[13] Abrao ALP, Leal SC and Falcao DP. Salivary and serum cortisol levels, salivary alpha-amylase and unstimulated whole saliva flow rate in pregnant and non-pregnant. Rev Bras Ginecol Obstet. 2014;36(2):72-78.
[14] Jain K, Kaur H. Prevalence of oral lesions and measurement of salivary pH in the different trimesters of pregnancy. Singapore Med J. 2015;56(1):53-57.
[15] 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.
[16] Guidozzi F, Maclennan M, Graham KM, Jooste CP. Salivary calcium, magnesium, phosphate, chloride, sodium and potassium in pregnancy and labour. S Afr Med J. 1992;81(3):152-54.
[17] Rockenbach MI, Marinho SA, Veeck EB, Lindemann L, Shinkai RS. Salivary flow rate, pH, and concentrations of calcium, phosphate, and sIgA in Brazilian pregnant and non-pregnant women. Head & Face Med. 2006;2:44.
[18] Rio R, Azevedo Á, Simões-Silva L, Marinho J, Silva MJ, Sampaio-Maia B. The biochemistry of saliva throughout pregnancy. Medical Express. 2015;2(5):M150506.
[19] Merglova V, Hecovaa H, Stehlikovaa J, Chaloupka P. Oral health status of women with high-risk pregnancies. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2012;156(4):337–41.
[20] Vibhute N, Vibhute A, Pawar R, Redder P, Tengshe M, Daule R. Self Reported Oral Health, Self Care and Dental Attendance of Pregnant Women in India: A Postnatal Survey. IJCMR. 2016; 3(2):555-58

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